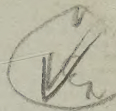


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HUGH ZACHARIAE



SKIN HISTAMINE

*Spectrofluorometric Studies on Normal
and Diseased Skin*



MUNKSGAARD COPENHAGEN 1965

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SKIN HISTAMINE

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and Diseased Skin*



MUNKSGAARD
COPENHAGEN 1965

Denne afhandling er i forbindelse med omstående tidligere publicerede
afhandlinger af det lægevidenskabelige fakultet ved Københavns
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København, den 19. maj 1965.

Harald Gormsen

h. a. dec.

Previous Publications

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- II. H. Zachariae: Skin histamine in urticaria. A spectrofluorometric assay. *Acta dermat.-venereol.* 1963, **43** : 214.
- III. H. Zachariae: Histamine in human skin. A spectrofluorometric assay. *Acta dermat.-venereol.* 1964, **44** : 219.
- IV. H. Zachariae: Skin histamine in erythema multiforme exudativum. *Acta dermat.-venereol.* 1964, **44** : 261.
- V. H. Zachariae: Influence of betamethasone, a synthetic steroid, on histamine in human skin. A spectrofluorometric assay. *Acta dermat.-venereol.* 1964, **44** : 330.
- VI. H. Zachariae: Polymyxin B, a potent releaser of histamine in human skin. *Acta dermat.-venereol.* 1964, **44** : 338.
- VII. H. Zachariae: Histamine in delayed skin reactions. Fluorometric determinations on patch tests. *J. invest. Derm.* 1964, **42** : 431.
- VIII. H. Zachariae: Histamine and mast cells in fetal skin. *Proc. Soc. exp. Biol.* 1964, **117** : 63.
- IX. H. Zachariae: Histamine in a transplantable mouse mastocytoma. *Proc. Soc. exp. Biol.* 1964, **117** : 283.
- X. A. Marckmann & H. Zachariae: Histamine in full thickness skin autografts of rat. *Proc. Soc. exp. Biol.* 1964, **117** : 705.
- XI. A. Marckmann & H. Zachariae: Histamine and mast cells in full thickness skin grafts of pig. *Acta chir. scand.* 1965, **129** : 12.
- XII. H. Wulff, H. Zachariae & K. Øhlenschläger: The leukocytic response to aseptic inflammation in urticaria pigmentosa. *J. invest. Derm.* 1965, **44** : 381.



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Preface

This survey of histamine in skin is based on a series of investigations carried out during the period 1962 to 1964 at the Connective Tissue Research Laboratory, Department of Skin and Venereal Diseases and at the Central Laboratory, Department of Clinical Chemistry, Rigshospital, University of Copenhagen.

My deepest thanks are due to professor *G. Asboe-Hansen*, M. D., director of the Connective Tissue Laboratory and head of the Department of Skin and Venereal Diseases, Rigshospital for his deep interest, constant encouragement and valuable criticism as well as for the complete scientific freedom I enjoyed.

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The experiments on pigs reported in one of the publications were performed at the Institute for Experimental Research in Surgery, University of Copenhagen. It is a pleasure to thank director *H. H. Wandall*, M. D. for the permission to undertake this investigation.

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For able and careful technical assistance during the experimental work and the preparation of the manuscript I thank Miss *Annelise Christensen*, Mrs. *Nina Christiansen*, Mrs. *Birgit Thorsen*, Miss *Kirsten Hasløv*, Mrs. *Wanda Juncker*, laboratory technicians, Miss *Wibeke Larsen*, medical student, Miss *Edith Andersen*, Miss *Lis Munk*, nurses, and Miss *Julie Liefeldt*, secretary. My thanks go also to Mr. *John Winther*, who prepared all photographs.

The studies were aided by grants from Mr. & Mrs. *Reinholdt W. Jorck's* Foundation, *F. L. Smidth & Co.'s* Foundation, *Johann* and *Hanne Weimann*, née *Seedorff's* Foundation, "Fonden til Lægevidenskabens Fremme" to dr. *A. Marckmann*, and USPHS Grant AM 06209-02 to dr. *G. Asboe-Hansen*.

Through her constant encouragement, unselfish support and great practical assistance, my wife, dr. *Eva Zachariae*, has a large share in these studies. I am gratefull to her and my children for their patience and resignation during the years of this work.

Fredensborg, April 1965.

Hugh Zachariae

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Introduction

Shortly after *Windaus & Vogt* in 1907 had synthesized histamine, the marked pharmacological effects of this chemical substance were discovered. In the following decade, histamine was shown to be present in most animal tissues, including skin. This fact led to research into the question of the physiological role of histamine, a problem which has not yet been solved. The relationship between histamine and allergy has been dealt with for years. Although an analogy between the pharmacological effects of histamine and those of anaphylaxis was pointed out by *Dale & Laidlaw* as early as 1910, histamine has not yet been proved responsible for the clinical manifestations in allergic conditions. The role played by histamine as an edema producing substance in connection with skin changes of various types in man has been made the subject of many authors. Few, however, have measured histamine. Histamine research until now has been carried out mainly on animals.

Previously the only suitable means of measuring histamine in tissues was bioassay (*Barsoum & Gaddum* 1935, *Code* 1937). The concept of the importance of histamine in human pathology, however, stimulated the search for increasingly sensitive methods (*Lubschez* 1950, *Lowry*, *Graham*, *Harris*, *Priebat*, *Marks & Bregman* 1954).

The spectrofluorometric assay (*Shore*, *Burkhalter & Cohn* 1959) has made possible the measurement of histamine in small skin biopsies. It was natural, therefore, to investigate the levels of skin histamine in normal and diseased skin. The present experiments were undertaken in an attempt to elucidate the significance of changes in skin histamine and to afford a survey of the role of histamine in skin.

Chapter I

Chemistry and Metabolism of Histamine

Histamine, β -imidazolethylamine (Fig. 1), is a well recognized, normal constituent of many mammalian tissues. It was synthesized as early as 1907 by *Windaus & Vogt* and later identified as a potent pharmacological agent. As it is partly responsible for the actions of aqueous extracts of ergot (*Barger & Dale* 1910 a), it was formerly named ergamine or ergotidine.

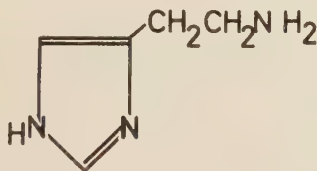


Fig. 1.

Histamine, β -imidazolethylamine.

Histamine is readily soluble in water, slightly soluble in alcohol and almost insoluble in ether and chloroform. In solution, it behaves as a dibasic molecule, and in its adsorption, it generally follows the pattern of other organic bases (*Code & McIntire* 1956). When the solutions are acid, histamine is stable even at boiling temperatures (*Barger & Dale* 1910 b). Condensation products are formed with several chemical substances; most of the older and some recent chemical methods for quantitative determination of histamine depend on such a coupling. In the diazo (*Lubschez* 1950) and dinitro-fluorobenzene methods (*Lowry, Graham, Harris, Priebat, Marks & Bregman* 1954), after some purification, the condensation products are measured spectrophotometrically. In the present method (*Shore, Burkhalter & Cohn* 1959) the condensation product with ortho-phthalaldehyde (OPA) is measured by spectrofluorometry. Besides the reaction with aromatic amines and aldehydes, conjugation also takes place between histamine and certain ions of heavy metals (*McIntire, White & Sproull* 1950).

Commercially, histamine is usually obtained either as histamine dihydrochloride or as histamine phosphate, containing 60 per cent and 35 per cent histamine base respectively.

Biologically, histamine is formed by enzymatic decarboxylation of histidine. Binding of exogenous histamine by tissues has not been demonstrated. Histidine decarboxylase is produced by a wide variety of organisms. Bacterial histidine decarboxylase is responsible for histamine in rotted ergot as well as in feces. The mast cells are the only mammalian *cells* in which the presence of a specific histidine decarboxylase has been demonstrated (Schayer 1956, Rothschild & Schayer 1959). Histidine decarboxylase activity, however, is also found in non-mast cell tissue (White 1959). According to Schayer (1961) there is evidence for the existence of an "inducible" form of histidine decarboxylase, of an unknown anatomical location producing "induced" (unbound) histamine. Apart from the specific histidine decarboxylase, a less specific histidine decarboxylase exists, which also acts on other aromatic amino acids, notably 3-4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan, a precursor of 5-hydroxytryptamine (serotonin). A typical example of this non specific enzyme, for which the term aromatic amino acid decarboxylase has been suggested (Lovenberg, Weissbach & Udenfriend 1962), is the histidine decarboxylase of guinea-pig kidney (Robinson & Shepherd 1961). Pyridoxal-5-phosphate is a known essential co-enzyme for histidine decarboxylase (Schayer 1959).

Nonspecific stress increases histidine decarboxylase activity (Schayer & Ganley 1959), and histidine decarboxylase activity can be increased by the administration of the histamine liberators compound 48 80 and polymyxin B. Numerous substances have been tested for their ability to inhibit histidine decarboxylase. The most potent inhibitor is α -methyldopa (Mackay & Shepherd 1960, Smith 1960, Udenfriend, Lovenberg & Weissbach 1960). Another potent histidine decarboxylase inhibitor is semicarbazide (Kahlson & Rosengren 1959). No substances, however, have been found yet, which are sufficiently specific to inhibit histidine decarboxylase without simultaneously inhibiting other enzymes.

The rate of histamine formation may be expressed in "histamine forming capacity", measured isotopically in minced tissues or cell suspensions incubated *in vitro* with small, physiological concentrations of histidine (Kahlson 1960). Nascent histamine is a newly introduced term implying increased formation of histamine in rapidly growing tissues (Kahlson 1962).

It is generally agreed that histamine exists in the body in three forms: free histamine, bound histamine, and conjugated histamine (see Code & McIntire 1956). The two first mentioned forms are the most important. In urine, however, the third form, which is acetylhistamine, regularly comprises the major component, its output being more related to dietary than metabolic factors (Mitchell & Code 1954). Also in feces it can be found in considerable amounts (Urbach 1949). In tissues it is split into free histamine only by such energetic measures as boiling in presence of acid (Rosenthal & Tabor 1948).

It is in its free form that histamine is able to produce its effects, but normally, very little free histamine is present in the fluids and intercellular spaces of the body (*Emmelin* 1945). Almost all the histamine which is determined consists of bound histamine. The binding, however, is weak and customarily partly or completely broken by homogenization and severed completely by mild acid condition or by common protein precipitants (*Code & McIntire* 1956). Most bound histamine is found within the mast cell (*Riley & West* 1952, *Riley & West* 1953) and in human mastocytosis large amounts of histamine have been demonstrated in the skin (*Zachariae* 1963 b) and urine (*Brogren, Duner, Hamrin, Pernov, Theander & Waldenström* 1959, *Demis, Walton & Higdon* 1961). The mechanism of the binding is not well understood. It has been suggested that histamine occurs rather firmly bound to proteins or lipoproteins (*Rocha e Silva* 1940). A binding by lecithin has also been proposed (*Lindahl* 1955). However, histamine can be removed from the skin by relatively mild procedures which do not suffice to break firm chemical bonds. A reasonable explanation could be that histamine is held in the mast cell by a fairly weak ionic linkage to macromolecules like heparin (*Sanyal & West* 1956). Such a complex has been shown *in vitro*, and the morphological characters and staining properties of the complex resemble those of mast cell granules.

Under normal conditions after formation and binding, intracellular histamine is released slowly from the tissues (*Schayer* 1952). Histamine release may also take place rapidly after stimuli of chemical or physical nature. It is suggested that the release processes require energy (*Uvnäs* 1961). This may be derived from glucose metabolism (*Diamant & Uvnäs* 1961, *Diamant* 1962).

The chemical agents producing histamine release are numerous. Among these are quaternary salts as d-tubo-curarine and laudexium, compounds as morphine, codeine, papaverine, pethidine, atropine and strychnine, depressor drugs as apresoline and priscol, sympatomimethic agents as amphetamine, tyramine and phenylethylamine, chemotherapeutic agents as stilbamidine and propamidine, certain antibiotics, particularly polymyxin B, also phenylbutazone and even antihistaminics. Extracts of *ascaris suis*, venoms and certain macromolecular colloids, such as acacia, polyvinyl pyrrolidine, dextran and starch may also release histamine. Certain enzymes are known to produce histamine release, int. al. trypsin and lecithinase A. Serotonin has been found to release histamine *in vitro*. The subject histamine release by chemical action has been exhaustively reviewed by *Paton* (1957) (see also *Arunlakshana* 1953, *Feldberg & Smith* 1953, *Walton, Richardson & Thompson* 1959, *Uvnäs* 1961, *Bray & van Arsdell* 1961, *Amann* 1962).

Histamine release due to physical tissue injury has been found after burns (*Rose & Browne* 1942, *Behrman, Schelling & Hartman* 1945), X-radiation

(Lasser & Stenström 1954, Eisen & Wilson 1957), trauma (see chapter V) and in certain individuals after cold or ultraviolet light (see Baer 1956).

The release of histamine may be effectively blocked by iodobenzoate, cyanide, dinitrophenol and diisopropyl fluorophosphate (Uvnäs 1958, van Arsdel & Bray 1961). The inhibitors of histamine release now available, however, are not of clinical value, owing to their toxicity (van Arsdel & Beall 1960).

Within recent years a serum phenomenon termed "histaminopexy" has been described. In the experiments of Parrot & Laborde (1953) normal human serum inhibited the histamine-induced contraction of the isolated guinea-pig ileum. This inhibition was reduced or absent when serum was used from persons considered belonging to an "allergic" group of patients. Others have also observed such individual differences in histamine "fixation" (Sidi, Reinberg, Hincky & Bourgeois-Spinasse 1958, Tappeiner, Tirschek & Wodniansky 1958). In cases with decreased histaminopexy, released histamine should be "destroyed" at a slower rate and therefore be able to produce more severe reactions. Still the phenomenon is difficult to interpret. Direct binding of histamine to serum proteins can not be demonstrated (Kaplan & Davis 1953).

When injected subcutaneously or intravenously histamine produces both local and systemic effects. When administered by mouth only little effect comes about, because histamine is destroyed in the digestive tract. The most prominent local effects are smooth-muscle contraction (Barsoum & Gaddum 1935) and increased vascular permeability, mainly of the venules (Majno & Palade 1961). The increased permeability is followed by an edema. In the dermis it may occur as an acute urticarial weal (Lewis & Grant 1924). In man, most of the observable systemic effects of histamine are circulatory. The earliest symptom is hot flushing. The cardiac rate increases (Weiss, Robe & Ellis 1932), a transient arterial hypotension is induced (Beall & van Arsdel 1960), the rate of blood flow may be increased (Stead & Warren 1944) and a consistent increase in the size and pulsation of the cerebral arterioles has been demonstrated (Weiss & Lennox 1931). Electrocardiographic changes may be found including sinus tachycardia, T-vector shifts and premature ventricular contractions (Peters & Horton 1944). Besides these circulatory effects bronchoconstriction has been found in experimental animals (Dale & Laidlaw 1910). Gastric secretion is stimulated (Best & McHenry 1931, Weiss, Robb & Ellis 1932), and vomiting and diarrhea may occur (Dale & Laidlaw 1910). Most of these local and systemic effects are found after the administration of the histamine liberator polymyxin B (Zachariae 1964 a, c) (see also chapter XII). It has also been shown that systemic effects, comparable with those of histamine, may occur in urticaria (Horton & Brown 1929) and in urticaria factitia (Lewis & Harmer 1927,

Kalk 1929) (see chapter VIII). All local and most of the systemic effects, except the action on the gastric mucosa, may be reduced by antihistaminic drugs (see *Feinberg, Malkiel & Feinberg* 1958). However, as mentioned above, antihistaminics may occasionally act as histamine releasers (*Arunlakshana* 1953). Lately, it has been shown that, depending upon their concentration, antihistaminics act in three different ways: by competitive inhibition of histamine, by destroying mast cells and releasing histamine, and by preventing mast cell damage and histamine release in anaphylaxis (*Mota & Dias da Silva* 1960).

The degradation of histamine intrinsically released has not been followed directly. Instead the metabolism of histamine has been studied through injection of ^{14}C -histamine intradermally and examining the metabolites in the urine (*Schayer & Cooper* 1956). The following substances have been found: histamine, methylhistamine, imidazole acetic acid, methylimidazole acetic acid, and acetylhistamine. Much of the imidazole acetic acid, however, is conjugated with ribose (*Tabor & Hayaishi* 1955). Methylation of histamine followed by oxidation of the formed methylhistamine, and oxidation of histamine into imidazole acetic acid seem to be the main metabolic pathways. Only a very small per cent of injected histamine is excreted in the unchanged form and still less as acetylhistamine. The processes are probably mediated by the enzymes diamine oxidase (*Tabor* 1951) and imidazole-N-methyl transferase (*Lindahl* 1958). Also a xanthine monoamine oxidase (*Schayer & Kobayashi* 1956, *Lindell* 1958) seems to participate. The relative importance of these enzymes varies in different species. In the rat, diamine oxidase (histaminase) is the major "histaminedestroying" enzyme (*Schayer* 1953). When diamine oxidase is inhibited by aminoguanidine, most injected or liberated histamine is excreted unchanged in the urine (*Schayer, Kennedy & Smiley* 1953). In the cat and dog, methylation is the principal route of inactivation (*Schayer, Kennedy & Smiley* 1953). In man both routes may be important (*Schayer* 1956, *Lindell, Nilsson, Schayer & Westling* 1958).

Studies with ^{14}C -histamine has also revealed that intravenously injected histamine disappears rapidly from the blood stream. The plasma has been found to be cleared of histamine radioactivity in few minutes. Seventy per cent or more of the administered radioactivity was recovered from the urine within twenty four hours, and all of it within forty eight hours (*Beall & van Arsdell* 1960).

Chapter II

Methods

The quantitative determination of histamine always requires two steps, the initial step devoted to the purification and the final stage to the assay of histamine. The assay may be accomplished by biological or chemical means, the latter involving either spectrophotometry or spectrofluorometry. In the author's studies all determinations were carried out by the spectrofluorometric method of assay, originally devised by *Shore, Burkhalter & Cohn* in 1959.

The histamine determinations were performed on approximately 30 mg of skin. From human skin and pig skin the subcutaneous fat was mechanically removed by knife or scissors. The human skin samples were normally either removed during operation under local anesthesia with Leostesin® (Lidocain NFN) or by punch biopsy under local freezing anesthesia with ethyl-chloride. The influence of local anesthesia on histamine determinations, under the circumstances used in this study, seems to be negligible. It was tested in eight cases, where biopsies were performed without anesthesia as well as during local anesthesia with 2 per cent lidocaine and under local freezing anesthesia with ethyl-chloride (table 1). General anesthesia was employed in a few cases, where human skin samples were removed from the head or hand during operations (*Zachariae* 1964 b). General anesthesia with ether was used in the studies on experimental animals.

In the investigations on rodents, if nothing else stated (*Marckmann & Zachariae* 1964), the determinations were carried out on samples which included subcutis and panniculus carnosus, the equivalent of the human platysma. This was done in order to compare skin histamine values with the results of other workers (table 7).

Almost all¹⁾ determinations were made on defatted and dried skin. Defat-

¹⁾ In the first paper published "Skin histamine in urticaria pigmentosa" (*Zachariae* 1963 b), histamine determinations were made on fresh skin. However, in these cases, skin histamine contents were so great that possible errors related to sebaceous glands, remaining subcutaneous tissue, or variations in water content, may be considered as unimportant.

Table 1

Comparison of histamine determinations performed on skin from the same body area removed either without anesthesia, during local anesthesia with 2 per cent lidocaine, or under local freezing anesthesia with ethyl-chloride

Case no.	Skin histamine content μg per g dried defatted skin		
	No anesthesia	Local anesthesia	
		Lidocaine	Ethyl-chloride
1.....	19.0	29.7	20.8
2.....	31.8	38.5	37.5
3.....	19.5	27.0	23.8
4.....	43.7	30.1	31.4
5.....	42.7	32.3	37.5
6.....	23.0	22.5	24.6
7.....	21.9	24.7	18.3
8.....	15.6	12.9	12.5
Mean.....	27.2	27.3	25.8
Standard deviation.....	± 11.0	± 7.6	± 9.0
Standard deviation of the mean.....	± 3.9	± 2.7	± 3.2

ting procedures have not been used by other workers. However, such procedures are important, to avoid errors related to sebaceous glands, and because small amounts of subcutaneous tissue may be present in spite of an apparently radical removal.

Defatting was carried out either with ether or with ether and acetone. Histamine is almost insoluble in ether and has very little solubility in acetone, so that it remains in the tissue. In order to verify this, under the experimental conditions employed, histamine determinations were performed on six skin extracts, prepared by shaking approximately 30 mg of normal human skin with 15 ml of ether or acetone for three hours. No histamine was detected in these extracts.

The defatted tissue was lyophilized for 48 hours at room temperature under a pressure of 2 mm Hg. It is important to calculate values on a dry weight basis, especially when determining an edema-producing substance like histamine. This has not in all instances been done by other workers.

Because the assay measures free histamine, the bound must be converted to the free form. This was accomplished by homogenization in perchloric acid. A motor-driven glass homogenizer was used. In order to control the homogenization six skin samples were homogenized for 10 minutes with 0.4 N perchloric acid. The homogenate was allowed to stand for 10 minutes and then centrifuged. A 4 ml aliquot of the supernatant was analyzed for histamine. The remaining material was washed twice with redistilled water,

homogenized for another 10 minutes, allowed to stand for 10 minutes and then centrifuged. Again a 4 ml aliquot was analyzed for histamine, but no histamine was found (table 2).

Table 2

Skin histamine levels in six skin samples (average wet weight 26.5 g) and residual amounts of histamine in the homogenates after extraction with 0.4 N perchloric acid and a double wash with redistilled water

	Skin histamine ($\mu\text{g/g}$)	Residual amount of histamine ($\mu\text{g/g}$)
Sample I	30.9	0
Sample II	29.4	0
Sample III.....	26.7	0
Sample IV.....	31.2	0
Sample V	27.4	0
Sample VI.....	21.1	0

The homogenization in perchloric acid also represents the first steps in the purification, involving an extraction and a protein precipitation. After this the precipitated material is removed by centrifugation, and a butanol extraction follows. To a 4 ml aliquot of the supernatant is added 1.5 g of solid NaCl, 0.5 ml 5 N NaOH and 10 ml butanol. The salt concentration greatly favors the extraction of histamine from the aqueous solution (*Code & McIntire* 1956), while the alkalinity is necessary to separate histidine and histidylhistidine from histamine. Histidine and histidylhistidine give rise to false reactions, but are not extracted by butanol from an alkaline solution (*Shore & co-workers* 1959). After shaking for 5 minutes and centrifugation, the aqueous phase is removed and the butanol phase is washed with 5 ml NaCl-saturated 0.1 N NaOH to remove any residual amounts of histidine. To check the separation of histidine from histamine, histidine was added to three different skin samples. The recovery of histidine in the butanol phase was investigated and found to be 0 per cent (table 3), despite the fact that

Table 3
Recovery of histidine

	Fluorescence intensity (meter readings)	Recovery of histidine
Sample I.....	45.4	
Sample I + 20 μg histidine...	45.3	0
Sample II.....	26.5	
Sample II + 20 μg histidine..	26.5	0
Sample III.....	21.2	
Sample III + 50 μg histidine .	21.1	0

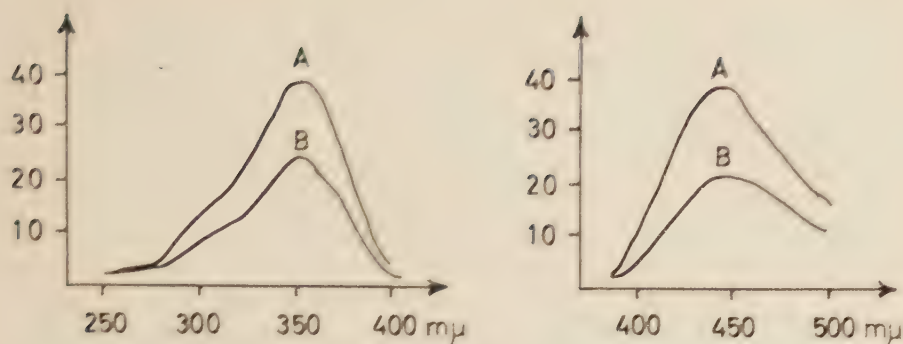


Fig. 2.

Excitation (left) and fluorescence (right) spectra of the fluorophore resulting from ortho-phthalaldehyde treatment of histamine from a skin homogenate (approximately 9 mg skin from a patient with urticaria pigmentosa) (A) and from authentic histamine (5 μg) (B). The ordinates are the fluorescence intensities expressed in arbitrary units, meter-readings. The abscissae are wave-lengths in mμ.

the same amounts of histidine after condensation with ortho-phthalaldehyde give a very strong fluorescence (Zachariae 1963 a).

Histamine is returned to aqueous phase by shaking an 2 ml aliquot of the butanol phase with 15 ml heptane and 4.5 ml 0.1 N HCl. The solubility characteristics of histamine are such that in the initial extraction about 92 per cent of the histamine is extracted into butanol. After the additional wash with NaCl-saturated NaOH about 25 per cent of the histamine remains (Shore & co-workers 1959). To correct for these facts, standards are prepared by carrying known amounts of histamine added to 0.4 N perchloric acid through the procedure.

The fluorometric assay is based upon the fact that the condensation product of histamine and orthophthalaldehyde (OPA) gives a very strong fluorescence with a characteristic fluorescence spectrum (fig. 2). The condensation is postulated to be as shown in fig. 3. Shore & co-workers (1959) maintain that this fluorophore is extremely specific, as alkylation of either of the nitrogen atoms in the imidazole ring or of the side chain blocks the

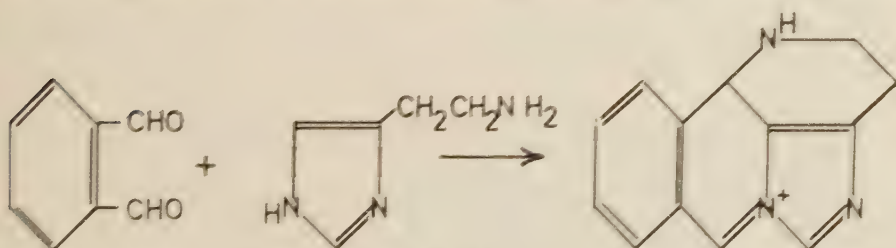


Fig. 3.

The condensation of histamine with ortho-phthalaldehyde.

reaction, and because histamine treated with metaphthalaldehyde or ortho-phthalaldehydic acid produces no fluorescence. They also mention that only histidine and histidylhistidine produce fluorophores spectrally similar to that of histamine when condensed with OPA.

Two ml of the aqueous solution is used for the fluorometric assay, while another 2 ml may be used to obtain the blank values of reagents plus skin²⁾. As the condensation with OPA only takes place in an alkaline solution (Shore & co-workers 1959) the 2 ml aliquot is transferred to a test tube containing 0.4 ml of 1 N NaOH followed by addition of 0.1 ml OPA-reagent³⁾. These are mixed immediately, and after 3½ minutes $\pm 1/2$ minute the fluorophore is stabilized by the addition of 0.2 ml 3 N HCl. The fluorescence is then measured in a spectrofluorometer⁴⁾. The fluorescence intensity is stable for one half hour.

The fluorescence blanks of reagents plus skin were determined by omission of the condensation step. This was accomplished by repeating the described procedure with the other 2 ml aqueous solution, but reversing the order of addition of OPA and 3 N HCl. This could be done since the condensation reaction does not proceed in acid environment. The reagent plus skin blanks were used for subtraction from the fluorescent reading of the sample. The reagent blanks, which were obtained by carrying 4 ml 0.4 N perchloric acid through the procedure, were used for correction of the standards.

Originally⁵⁾ the fluorescence was measured at 445 m μ resulting from activation at 355 m μ from a xenon lamp as the maximum fluorescence intensities were obtained by the use of these wave-lengths (fig. 2 a). Later, however, 369 m μ from a mercury lamp was preferred, on account of the greater stability of this lamp. This could be done because almost identical fluorescence spectra were obtained in the two cases (fig. 4). It was then chosen to measure at 450 m μ in stead of 445 m μ , because 450 m μ has been used by most workers dealing with spectrofluorometric determinations of histamine, and because any differences in fluorescence intensities were negligible (fig. 2).

In order to evaluate the method, the analyses were first carried out with known amounts of histamine added to 0.4 N perchloric acid throughout the procedure and a standard curve was drawn (fig. 5). Several trials were

²⁾ In the first two articles published, "Skin histamine in urticaria pigmentosa" (Zachariae 1963 b) and "Skin histamine in urticaria", (Zachariae 1963 c) only blank values of reagents were used.

³⁾ Ortho-phthalaldehyde, 1 per cent in methanol.

⁴⁾ In the authors studies: model 104243 B, Farrand Optical Co., New York, or Zeiss spectrophotometer PMQ 11 with fluorescence attachment ZFM 4.

⁵⁾ In the studies "Skin histamine in urticaria pigmentosa" and "Skin histamine in urticaria", (Zachariae 1963 b, c).

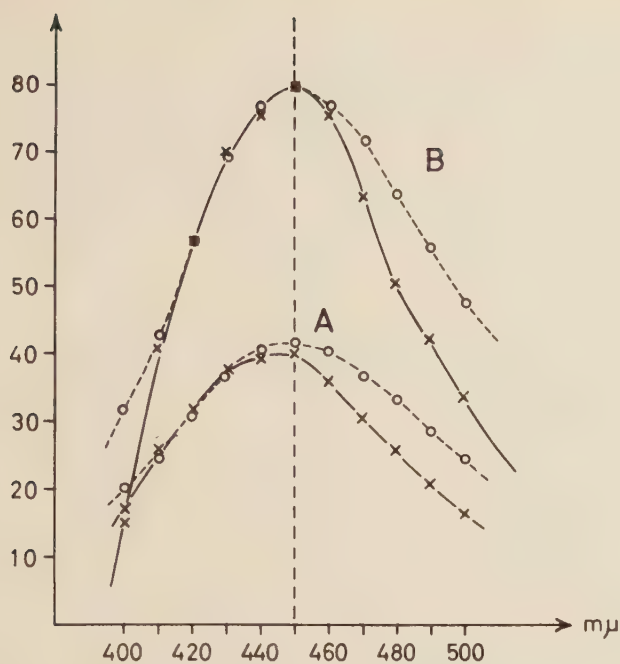


Fig. 4.

Fluorescence spectra of the fluorophore resulting from ortho-phthalaldehyde treatment of (A) apparent histamine from human skin and (B) authentic histamine using excitation wave-lengths of 355 mμ ---- and 369 mμ ———.

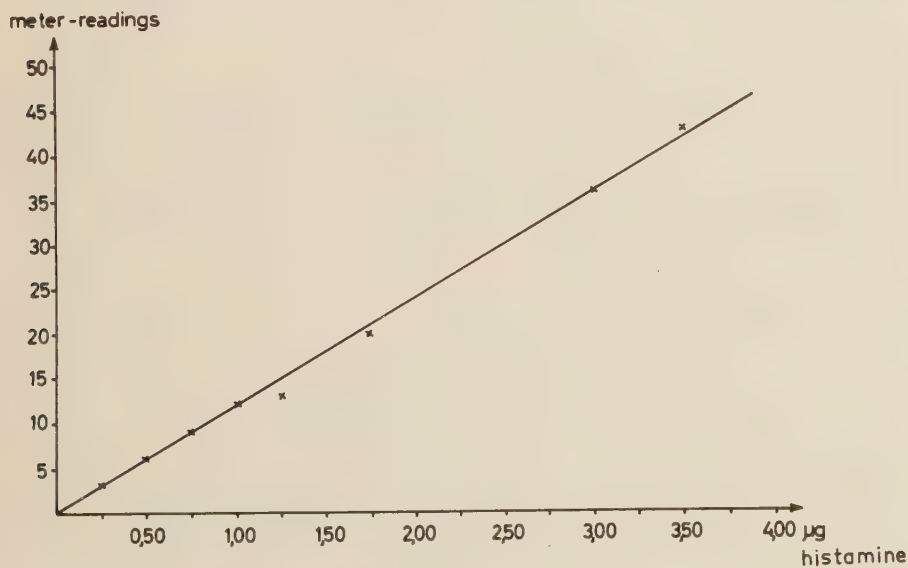


Fig. 5.

Fluorescence intensity of histamine in aqueous solutions. The intensity is expressed in arbitrary units, meter-readings minus meter-readings of reagent-blank.

Table 4
Recovery of histamine

	Histamine μg	Recovery of added histamine	
		μg	per cent
Sample A	0.72		
Sample A + 0.5 μg histamine.....	1.20	0.48	96
Sample A + 0.5 μg histamine.....	1.19	0.47	94
Sample B	0.09		
Sample B + 0.5 μg histamine.....	0.52	0.43	86
Sample B + 1.0 μg histamine.....	1.19	1.10	110
Sample B + 1.5 μg histamine.....	1.64	1.55	103
Sample B + 2.0 μg histamine.....	2.03	1.94	97
Sample B + 2.5 μg histamine.....	2.53	2.44	98
Sample C	0.40		
Sample C + 0.5 μg histamine.....	0.83	0.43	86
Sample C + 1.0 μg histamine.....	1.35	0.95	95
Sample C + 1.5 μg histamine.....	1.86	1.46	97
Mean.....			96.2
Standard deviation			- 7.1
Standard deviation of the mean.....			± 2.2

made, all of which showed the fluorescence intensity to be proportional to the concentration of histamine within the range examined.

The recovery was also investigated (table 4) and found to range from 86 to 110 per cent, with an average of 96.2 per cent.

To support the identification of histamine, excitation and fluorescence spectra of the fluorophore resulting from OPA treatment of apparent histamine from skin (fig. 2) were found identical with those of the OPA-condensation product of authentic histamine (fig. 2). Solubility characteristics of apparent skin histamine were also investigated and compared with those of authentic histamine (table 5). Aliquots of butanol extracts of skin

Table 5
Comparative partition ratios of authentic histamine and
apparent histamine of skin

Buffer pH	Per cent remaining in organic phase	
	Authentic histamine	Apparent histamine
8.5	15.8	14.3
10.0	35.7	39.9
12.0	46.8	42.9

homogenates and of water to which authentic histamine had been added were shaken with equal volumes of three different buffers. The relative amounts of authentic or apparent histamine remaining in the organic phase were measured and found to correspond.

The standard error of the method was investigated and calculated to be $\pm 0.2 \mu\text{g/g}$, when comparing histamine levels in six equal portions of the same homogenate (table 6). The standard deviation was $\pm 0.6 \mu\text{g/g}$ (table 6).

Table 6
Calculated histamine levels in six equal portions of the same skin homogenate

No.	Histamine ($\mu\text{g/g}$)
1.....	23.8
2.....	23.7
3.....	24.4
4.....	24.8
5.....	25.3
6.....	24.9
Mean.....	24.5
Standard deviation.....	± 0.6
Standard deviation of the mean.....	± 0.2

In table 7 are shown skin histamine levels of various species as measured by the OPA fluorescence assay, compared with results by other investigators. The levels generally are in agreement with those obtained by other technics.

In comparison with the spectrophotometric methods of determining skin histamine and bio-assay, the fluorometric assay has a considerable advantage by its greater simplicity. The procedure is also less time-consuming. Moreover, the almost identical activation and fluorescence spectra, as well as the almost identical solubility characteristics of apparent histamine from skin and authentic histamine indicate a high specificity. The method shows a great sensitivity and concentrations as low as $0.5 \mu\text{g/g}$ can be assayed in small skin biopsies weighing approximately 30 mg (wet weight).

It is concluded that the method offers a simple, accurate, time-saving, and sufficiently specific assay of skin histamine, bound plus free histamine. Acid hydrolysis was not performed in the present studies, so the amount of conjugated histamine or acetylhistamine has not been determined. In normal human abdominal skin, acid hydrolysis should produce a three-fold increase in histamine levels (*Johnson 1957*).

Table 7

Skin histamine levels of various species determined by spectrofluorometry, compared with results of other investigators using other technics.

Origin of skin	Skin histamine ($\mu\text{g/g}$ wet skin)	
	Spectrofluorometry	Other methods
Human chest skin.	8.6	8.8 (Bio-assay, Feldberg & Loeser, 1955) 8.2 (Spectrophotometry, Johnson, 1957)
Human abdominal skin.....	5.1	7.8 (Bio-assay, Feldberg & Loeser, 1955) 7.6 (Spectrophotometry, Johnson, 1957)
Back skin of guinea-pig.....	1.3	2.9 (Bio-assay, Feldberg & Miles, 1953) 3.9 (Spectrophotometry, Johnson, 1956)
Back skin of rat...	25.2	38.1 (Bio-assay, Hvidberg & co-workers, 1961) 41.8 (Spectrophotometry, Johannesson & Norn, 1963)
Back skin of mouse	37.5	40 (Bio-assay, Perry, 1956)
Back skin of rabbit	2.3	2.0 (Bio-assay, Riley, 1956*)

*) Abdominal skin of rabbit.

Where nothing else is stated, the values represent histamine base per gram defatted, dried tissue.

Where histologic studies have been performed, the biopsies were fixed in a 4 per cent aqueous lead subacetate solution for 24 hours and stained with 0.5 per cent aqueous solution of toluidine blue, which rather selectively stains acid mucopolysaccharides metachromatically (*Holmgren* 1938).

The leukocytic response to aseptic inflammation in urticaria pigmentosa (*Wulff, Zachariae & Øhlenschläger* 1964) was studied using *Rebuck & Crowley's* "skin window" technic (1955). The "skin window" was made by scraping away the epidermis. The excoriation was covered by a coverslip, which was changed at three-hourly intervals for twelve hours. In contrast to the original procedure no foreign protein was applied to the excoriation. Leukocytes emigrated to the undersurface of the coverslip, which, after removal, was dried and stained using the May-Grünwald-Giemsa method.

Mean values are given together with standard deviations and standard deviations (standard errors) of the mean. Comparisons of different groups were made by Student's t-test (*Fischer* 1944). A p value of 0.05 or less was considered significant.

Chapter III

Histamine in Normal Skin

Species variations in tissue histamine have been known for several years (see *Feldberg* 1956). The present author investigated the histamine levels in back skin of man, pig, rabbit, guinea-pig, rat and mouse (table 8). Skin histamine was found high in rat and mouse, less in man and pig, which had almost the same amounts of histamine in their back skin, while the back skin of the guinea-pig and the rabbit had relatively low histamine levels. Data for dog and cat skin have been submitted by *Riley* (1959), and for monkey and ferret skin by *Perry* (1956). The abdominal skin of the dog has approximately the same amount of histamine as human abdominal skin. The cat tends to have skin histamine levels intermediate between man and rat. The monkey has a lower skin histamine level than man, but not as low as the guinea-pig. Ferret skin ranges in histamine content with guinea-pig skin.

Striking regional differences in skin histamine were detected by *Feldberg & Miles* in 1953 in the guinea-pig. This work was based on bio-assay. The results were later confirmed with the dinitrofluorobenzene method by *Johnson* in 1956. Both studies revealed high values in the face, and *Feldberg* and *Miles*, who studied a larger number of skin areas, found also high histamine levels in the skin of the ears, the nipples and the perivaginal

Table 8

Species variations in skin histamine

Histamine levels in dried and defatted back skin of man, pig, rabbit, guinea-pig, rat and mouse

Species	Number of individuals investigated	Skin histamine ($\mu\text{g/g}$)	Standard deviation ($\mu\text{g/g}$)
Mouse.....	12	197.6	± 43.9
Rat.....	6	93.0	± 25.9
Pig.....	9	28.2	± 12.5
Man.....	10	26.4	± 10.6
Rabbit.....	8	8.0	± 2.7
Guinea-pig.....	7	4.1	± 2.0

area. Comparative studies of various animals have shown, that whether the general level of skin histamine is high or low, the ears, face and paws show the relatively highest histamine values (see *Feldberg* 1956). In 1957, *Johnson*, in a study performed on autopsy material (thirteen cases of sudden traumatic death) could confirm that regional differences were also to be considered in human skin. *Zachariae* (1964 b) studied skin histamine in

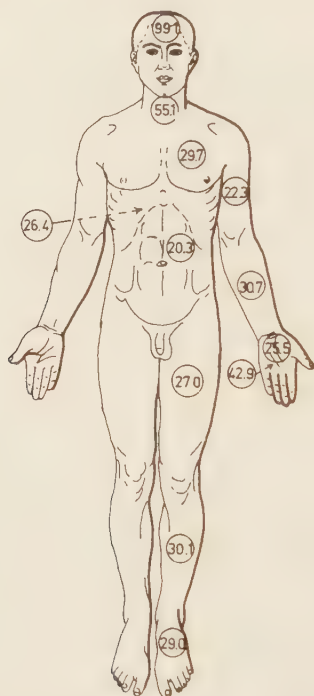


Fig. 6.

Average histamine levels in various skin areas. The values are μg histamine base per g dried defatted skin.

patients without skin diseases and found regional variations following the same pattern as in experimental animals (fig. 6). The highest levels occurred on the eyelids, cheeks and upper lip. This may have bearing upon the common clinical observation that these areas are the most liable to react with swelling following various sorts of injury. High values were also found in other areas of the head, neck and dorsum of the hands. The determinations from the skin of the head (excluding the eyelids and the ears) showed an almost five times higher average histamine value than the determinations from the abdominal skin. One of the determinations from the eyelids was ten times higher than the average level in abdominal skin. The difference between the values for the head skin and that of the abdomen was signif-

Table 9

Ratio of mean values of histamine in various areas of human skin
to the mean value of abdominal skin

Skin region	Number of determinations	Ratio
Eyelids	2	9.3
Head*)	10	4.9
Ears	2	4.1
Neck	6	2.7
Hand, dorsum	10	2.1
Chest	10	1.5
Forearm	10	1.5
Leg	10	1.5
Foot, dorsum	10	1.4
Back	10	1.3
Hand, palm	10	1.3
Thigh	10	1.3
Upper arm	10	1.1
Abdomen	10	1.0

*) Skin of eyelids and ears not included.

icant ($p < 0.01$). In table 9 are shown ratios of mean values of histamine in various areas of human skin to the mean value of abdominal skin.

Individual variations are great in normal human skin. Studied in back skin from 10 "normal" persons, 6 women and 4 men, *Zachariae* (1964 b) found histamine levels ranging from 11.7 $\mu\text{g/g}$ to 42.6 $\mu\text{g/g}$. The mean value was 26.4 ± 3.4 $\mu\text{g/g}$, the standard deviation was ± 10.6 $\mu\text{g/g}$. When studied in grossly normal skin from the back of 126 patients with various local skin diseases affecting other areas than the investigated, histamine levels ranged from 8.7 $\mu\text{g/g}$ to 52.3 $\mu\text{g/g}$. The mean value was 26.0 ± 0.9 $\mu\text{g/g}$, and the standard deviation ± 9.5 $\mu\text{g/g}$. Variations in skin histamine from the same area of the same person were studied in grossly normal back skin from a patient operated on for lumbar disc protrusion (*Zachariae* 1963 c and 1964 f). The mean value of 11 different pieces taken from a skin sample measuring 8 by 2 cm was 40.9 ± 1.7 $\mu\text{g/g}$, the standard deviation was 5.8 $\mu\text{g/g}$. The skin histamine levels varied from 31.8 $\mu\text{g/g}$ to 38.6 $\mu\text{g/g}$.

The data available of the influence of age on skin histamine have been rather confusing. *Riley & West* (1953) found an increase with age in the skin of the cat. However, *Rocha e Silva* (1940) obtained just the opposite result for the skin of rabbits; and in guinea-pigs, which were investigated by *Johnson* (1956) a definite trend towards decreasing skin histamine levels with age was demonstrated. *Moretti, Lojodice & Ena* (1963) studying histamine in retroauricular skin from children of varying age found a significantly lower value in the group aging from nine months to fourteen years than in the group aged three to seven months. The younger group

presented histamine levels in dry skin ranging from 21.37 $\mu\text{g/g}$ to 54 $\mu\text{g/g}$ with an average of 46.14 $\mu\text{g/g}$. The levels of the older group ranged from 16.86 $\mu\text{g/g}$ to 40.1 $\mu\text{g/g}$ with an average value of 30.24 $\mu\text{g/g}$.

Zachariae (1964 b, g) studied the influence of age in human fetal skin and in adult human skin. The investigations revealed a rise with increasing fetal age. Histamine did not appear in larger quantities until the fifth fetal month (fig. 9 page 40), although minor amounts were found sporadically from the end of the third fetal month. Histamine values of fetal back skin of four fetuses, 18 to 20 weeks old, were 19.2 $\mu\text{g/g}$, 9.3 $\mu\text{g/g}$, 12.7 $\mu\text{g/g}$ and 16.1 $\mu\text{g/g}$. The mean value was 14.3 ± 2.2 $\mu\text{g/g}$. The mean value of back skin from fetuses 18 to 20 weeks old was significantly higher than the mean value (1.08 ± 0.7 $\mu\text{g/g}$) of back skin from six fetuses 12 to 14 weeks old ($p < 0.0025$).

In the investigation on adult human skin, which included 125 determinations of skin histamine in grossly normal back skin from patients without skin diseases or with local skin diseases affecting other areas than the back, *Zachariae* (1964 b) found a moderate decrease with advancing age. The difference between the age group of patients more than 60 years old and the age group of patients less than 40 years old was significant ($p < 0.05$).

In the same study the possible influence of sex on skin histamine was investigated. No significant differences were found between the values of the women and those of the men.

In order to study which layer of the skin was richest in histamine (and mast cells) *Riley* (1959) subdivided samples of fresh abdominal skin from guinea-pigs, rabbits, dogs, cats, mice, rats, and humans into two layers, an inner and an outer layer, the inner layer consisting of dermis and the outer layer of epidermis plus a thin layer of dermis. *Riley* found that the cat and rat each have only one main concentration of histamine yet the two species differ in that the histamine in the cat's skin is chiefly in the outer layer, while in the rat mainly in the inner layer. Man and dog more or less follow the pattern of the cat having most of their histamine in the outer layer. In the mouse, guinea-pig and rabbit, however, the location of histamine is almost the same in the inner and outer layer of the skin. The present author studied the same matter in the skin of the pig, and found the highest concentration of histamine in the outer layer (table 10). This emphasizes that in man, pig, dog, cat and rat, when different skin samples should be compared, they must represent the same layers of skin.

In 1952, *Riley & West* reported a positive correlation between the qualitative estimated frequency of mast cells and the histamine content of various tissues, among these skin. In mast cells from the capsule of the ox-liver *Riley & West* (1953) found a concentration of approximately 25 $\mu\mu\text{g}$ per cell. The histamine determinations were performed by the biological method

Table 10

Histamine values in the outer and inner zones of pig skin

Case number	Histamine ($\mu\text{g/g}$)	
	Outer zone	Inner zone
1.....	30.7	2.0
2.....	27.0	3.6
3.....	30.7	3.5
Mean.....	29.4	3.0
Standard deviation.....	± 2.1	± 0.9
Standard deviation of the mean.....	± 1.2	± 0.5

of assay. *Graham, Lowry, Wahl & Priebat* (1955), using the dinitrofluorobenzene method, could confirm this correlation, and in dog skin they estimated the amount of histamine per mast cell to 7 μg . When the size of the mast cell was taken into account, *Graham* and coworkers found the intracellular concentration of histamine in dog skin to be 0.1 molar.

Zachariae studied the relationship between skin histamine and mast cells in human fetal skin (1964 g), in urticaria pigmentosa (1963 b), in experimental skin tumors of mice (see chapter X), and together with *Marckmann* in full thickness skin grafts of pigs and rats and fresh granulation tissue of pig skin (1964, 1965). The highest skin histamine values were, as expected, found together with an accumulation of numerous dermal mast cells in urticaria pigmentosa (see also chapter IV). In human fetal skin the appearance of more than a few faintly granulated mast cells coincided with the presence of larger amounts of cell-bound histamine in the fifth fetal month. In the fresh granulation tissue of the pig skin no histamine and no mast cells could be demonstrated. Only small amounts of histamine and few mast cells were found in full thickness skin grafts of pigs and rats in the early stages after the operation. This in contrast to a considerable amount of histamine and larger numbers of mast cells in the skin prior to the operation (see chapter V). Finally, it was possible to find a correlation between histamine and mast cells in experimental skin tumors of mice (see chapter V). Thus also spectrofluorometric studies support the idea that the histamine value of skin reflects its mast cell population and that mast cells are sources of cell-bound skin histamine. This brings the investigation on the influence of age on skin histamine in agreement with the observations of *Hellström & Holmgren* (1950), who in a statistically analyzed material found that the mast cells decrease in number with advancing age. Besides as mast cells also contain acid mucopolysaccharides of the heparin and hyaluronic acid

types (see *Asboe-Hansen* 1963 a), the present investigation also agrees with the studies of *Clausen* (1962 a, b) who reported a decrease in the content of acid mucopolysaccharides with advancing age.

A review of the literature, and the results of the present investigation demonstrate that certain important facts should be considered, when studying skin histamine; namely species differences and regional variations, the age of the individuals and the method of tissue extraction employed (see Chapter II). It must be emphasized, that large individual variations seem to exist, which should not be underestimated. It should also be noticed if the samples investigated are representative of the whole skin, i. e. include all layers down to the subcutaneous tissue, or only consist of a part of the skin. Anyone, who wants to study changes in skin histamine and is not aware of these factors is apt to draw wrong conclusions.

Chapter IV

Skin Histamine and Mastocytosis

Among the most striking examples of local aggregations of mast cells in skin are the lesions of urticaria pigmentosa, the skin manifestation of mastocytosis. Urticaria pigmentosa was discovered by *Nettleship* who in 1869 wrote an article describing a rare form of urticaria: "Chronic urticaria, leaving brown stains, nearly two years' duration". *Unna*, in 1896, described urticaria pigmentosa as a rare disease characterized by skin changes consisting of subepitheleal accumulations of mast cells. In the meantime these granular cells of the connective tissue had been discovered by *Ehrlich* (1877).

Lately it has been accepted that urticaria pigmentosa is neither very rare, nor strictly limited to the skin (*Berlin* 1955, *Meneghini & Hofmann-Mailand* 1960, *Niordson* 1962). There have been numerous reports on changes involving mast cell infiltration in liver, spleen, vessel walls and bone marrow (*Sagher, Cohen & Schoor* 1952, *Asboe-Hansen* 1953, *Asboe-Hansen & Kaalund-Jørgensen* 1956, *Deutsch, Ellegast & Nosko* 1956, *Asboe-Hansen* 1960).

Clinically, the skin condition manifests itself by the appearance of pigmented macules and papules, which develop into weals on stroking or other physical irritation. The fundamental studies on wealing which were carried out by *Lewis & Grant* (1924) might have indicated that histamine would be present in urticaria pigmentosa. Although, in 1929, *Dale* attributed to local histamine release the reactions so readily elicited of the foci of urticaria pigmentosa, for a long time the interest was focused on the sulphated mucopolysaccharide heparin. This was after *Holmgren & Wilander* (1937) had claimed that the mast cell is the site of formation or storage of this anti-coagulant. Clinical symptoms of heparin release, however, have only exceptionally been demonstrated in patients suffering from mastocytosis (*Urbach, Bell & Jacobson* 1955).

It is only lately that *Riley & West* (1952) (see also *Riley* 1959) when

studying mast cells and histamine in skin lesions of urticaria pigmentosa found considerable quantities of histamine, and a strong correlation between the amount of histamine and mast cells. This supported the view that the histamine value in skin varies directly with its mast cell content, and that histamine plays an important role in the pathogenesis of urtication. The histamine determinations reported by *Riley* (1959) ranged from 20.0 to 950.0 $\mu\text{g/g}$ skin (wet weight). Recently, several other workers have published high skin histamine values in urticaria pigmentosa (*Sjoerdsma, Waalkes & Weisbach* 1957, *Bloom, Dunér, Pernow, Winberg & Zetterström* 1958, *Lindell, Rorsman & Westling* 1961 a, *Stüttgen, Sturm & Brüster* 1962).

Zachariae (1963 b) reported three cases of urticaria pigmentosa with extremely high values of histamine in the skin. The values were 1033, 1042 and 2083 $\mu\text{g/g}$ (wet weight) in three girls aged 8, 11 and 10 months. Other patients investigated by the author show values ranging from 9.1 to 743.0 $\mu\text{g/g}$ (wet weight) (table 11). In all cases the clinical diagnosis was

Table 11
Skin histamine levels in urticaria pigmentosa*)

Case no.	Sex	Age (years)	Skin histamine ($\mu\text{g/g}$)	
			Urticaria pigmentosa lesions	Non-pigmented skin
1.....	F	$\frac{5}{12}$	191.2	15.9
2.....	M	$\frac{6}{12}$	743.0	80.5
3.....	F	$\frac{8}{12}$	1033.0	
4.....	F	$\frac{8}{12}$	248.5	20.2
5.....	F	$\frac{10}{12}$	2083.0**)	
6.....	F	$\frac{11}{12}$	1042.0**)	
7.....	M	$\frac{13}{12}$	356.0	18.8
8.....	M	$\frac{13}{12}$	88.0	
9.....	F	$1\frac{1}{2}$	636.8	
10.....	F	$1\frac{1}{2}$	650.0	
11.....	M	$1\frac{3}{4}$	396.0	33.2
12.....	M	$1\frac{3}{4}$	468.0	14.2
13.....	M	$1\frac{3}{4}$	631.3	12.1
14.....	F	12	13.3	5.1
15.....	M	13	34.1	12.8
16.....	F	18	17.9	9.5
17.....	M	36	12.5	
18.....	F	42	17.2***)	15.9***)
19.....	M	51	44.2	21.8
20.....	F	59	9.1	8.5

*) All skin samples were from the back with the exception of **) and ***). The values are μg histamine base per g fresh skin.

**) Skin samples from the chest.

***) Skin samples from the thigh.

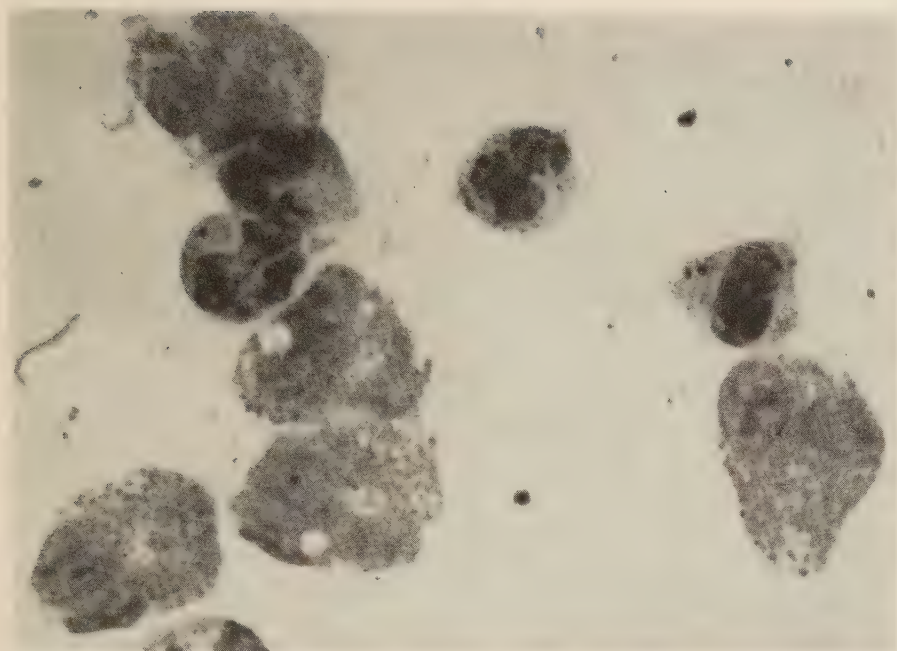


Fig. 7.

Eosinophils and neutrophilic granulocytes in a "3 hour skin window preparation" from a patient with urticaria pigmentosa. Staining: May-Grünwald-Giemsa.

confirmed by histological examinations of the skin lesions showing mast cell accumulation in the corium and melanin pigmentation of the basal epidermic cells (fig. 1). The highest values were found in the pigmented skin of the youngest individuals. A marked difference was found in skin histamine between the infants and the patients more than twelve years old (table 11). Although this is in agreement with the decrease in skin histamine normally found with advancing age (see chapter III), the difference is remarkably high and contrasts with the small differences found between the histamine levels of the non-pigmented skin of the two age groups (table 11).

Wulff, Zachariae & Øhlenschläger (1965) studied the leukocytic response to aseptic inflammation in lesions of urticaria pigmentosa. The leukocytes were sampled using *Rebuck & Crowley's* "skin window" technic (1955). The cellular exudate in preparations from five patients with urticaria pigmentosa differed considerably from that found in normal subjects (*Riis & Wulff* 1960, *Wolf-Jürgensen* 1962). A pronounced emigration of eosinophils and the presence of basophilic granules in many of the macrophages was observed in all cases. The eosinophils dominated the early preparations sampled three hours after the scraping of the epidermis (fig. 7) (table 12).

Table 12

The percentage of eosinophils in "skin windows" from 5 patients with urticaria pigmentosa in relation to blood-eosinophil counts and histamine determinations of urticaria pigmentosa lesions from back skin. Normally the eosinophils do not exceed 0.5 per cent in "skin windows" (Wolf-Jürgensen, 1962). The normal histamine content of back skin has been found to range from 11.7 to 42.6 μg per g dried defatted skin

Patient	Eosinophils in "skin window" at 3 hrs., per cent of total cell count	Blood eosinophils/ μl	Histamine content, $\mu\text{g/g}$ dried defatted weight
Boy, 12 months.....	49	413	1277
Boy, 2 years.....	41	694	2311
Girl, 2 years.....	16	175	2060
Girl, 2 years.....	11	225	1500
Boy, 13 years.....	10	200	106.8

Many cells were enmeshed in strands of a thready material, probably containing acid mucopolysaccharides (fig. II). In the following samples the percentage of macrophages increased (fig. 8). In all preparations the basophils remained few in number, less than 1 per cent, while the macrophages presented an unusual picture, due to the frequent occurrence of basophilic granules in the cytoplasm. The histamine determinations of the urticaria pigmentosa lesions were, as expected, highly increased, while leukocyte, differential and eosinophil counts were within normal limits, except in one patient, who showed a moderate increase in eosinophils.

It was suggested that the mechanical trauma at the onset of the experiments brought about mast-cell degranulation and histamine release. The free mast cell granules were gradually ingested by emigrating macrophages. It was also suggested that the release of histamine caused emigration of eosinophils (see later chapter IX).

High histamine values have also been demonstrated in mastocytomas of the dog, cow, horse, cat and mouse (Riley & West 1952, Dunn & Potter 1957, Sjoerdsma, Waalkes & Weissbach 1957, Riley 1959, Lindell, Rorsman & Westling 1959, Grof & Kelényi 1963). In a mouse with a transplantable mast cell neoplasm (Rask-Nielsen & Christensen 1963) Zachariae (1964 h) found 993 $\mu\text{g/g}$ histamine in dried and defatted skin compared with normal values in mouse skin ranging from 143.7 $\mu\text{g/g}$ to 276.4 $\mu\text{g/g}$. The primary tumour arose in a mouse that had been inoculated subcutaneously with leukemic tissue from a transplantable plasma cell leukemia. There was no visible growth of the inoculated material. Macroscopically the neoplasia appeared as white, opaque nodules in the mesentery. Microscopically, the primary and the transplanted tumours consisted of mast cells. The investigated mouse was from the first passage where tumour tissue

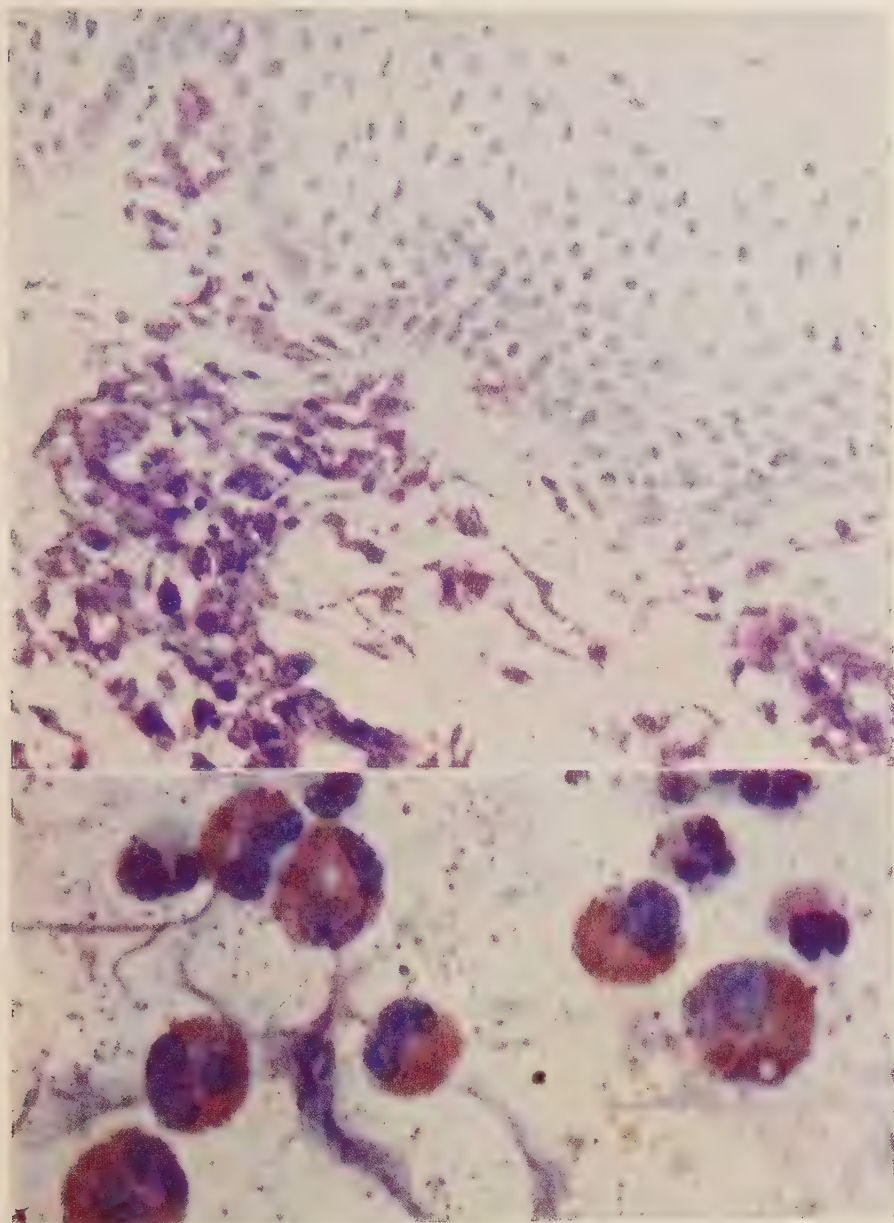


Fig. I.

Microphoto of urticaria pigmentosa skin. Note large amounts of mast cells in the corium and pigmentation of the basal epidermic cells. Staining: Toluidine blue, 0.5 per cent aqueous.

Fig. II.

A "3 hour skin window preparation" from a patient with urticaria pigmentosa. Eosinophils and neutrophilic granulocytes enmeshed in strands of a tready material, probably containing acid mucopolysaccharides. Staining: May-Grünwald-Giemsa.

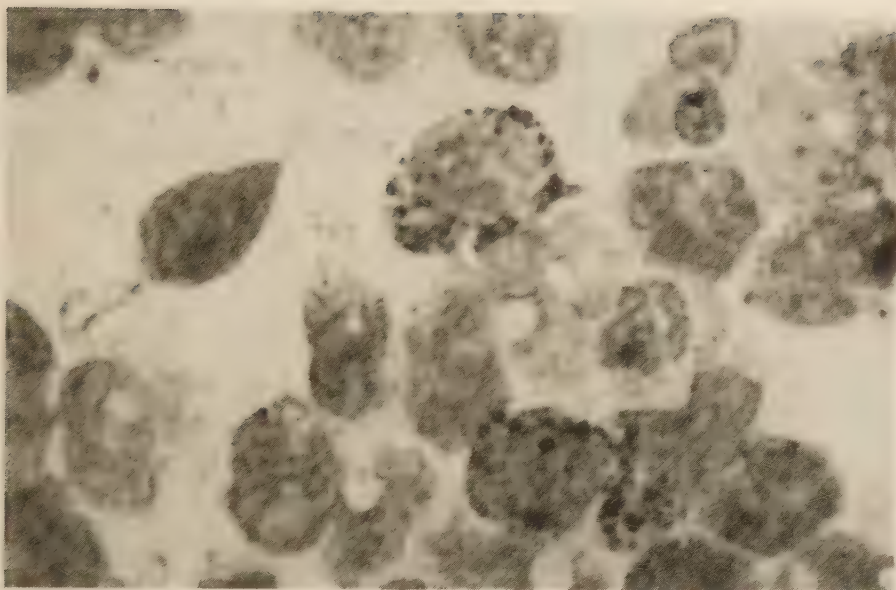


Fig. 8.

A "12 hour skin window preparation" from a patient with urticaria pigmentosa. The picture is dominated by macrophages, several of which containing phagocytized basophilic and eosinophilic granules. Staining: May-Grünwald-Giemsa.

had been inoculated intraperitoneally. The local growth consisted of soft, whitish, flabby tissue located to the mesentery. The histamine content of the tumour was 11.797 mg/g defatted tumour tissue. Otherwise, there is scarce information concerning the histamine content of the mouse mastocytoma. *Sjoerdma, Waalkes & Weissbach* (1957) found from 470 to 560 μ g/g in Dunn-Potter tumours, *Furth, Hagen & Hirsch* (1957) from 0.85 to 4.2 mg/g in Furth tumours, and *Grof and Kelényi* (1963), in transplantable Furth tumours, from 286 to 1114 μ g/g, all in fresh tumour tissue. *Cass, Marshall & Riley* (1952) found 1700 μ g histamine per g lyophilized Furth mastocytoma material.

In mice, the mastocytomas are exceptional in that they also contain substantial amounts of 5-hydroxytryptamine (serotonin) (*Furth, Hagen & Hirsch* 1957, *Cass, Marshall & Riley* 1952). Rat and mouse mast cells are known to hold 5-hydroxytryptamine besides histamine (*Rowley & Benditt* 1956, *Benditt, Holcenberg & Lagunoff* (1963). Human mast cells apparently do not contain 5-hydroxytryptamine (*Sjoerdma, Waalkes & Weissbach* 1957); but 5-hydroxytryptophan, a precursor of 5-hydroxytryptamine has been found in lesions of urticaria pigmentosa (*Stüttgen, Sturm & Brüster* 1962). If 5-hydroxytryptophan is released in the presence of a 5-hydroxytryptophan

decarboxylase, 5-hydroxytryptamine should appear, and in fact has been reported found in blister fluid from a patient with urticaria pigmentosa (*Braun-Falco* 1961). Other workers, however, have not been able to find evidence of any capacity of urticaria pigmentosa lesions to decarboxylate 5-hydroxytryptophan (*Birt, Hagen & Zebrowski* 1961, *Demis, Walton, Woolley, Wilner & McNeil* 1961).

Chapter V

Skin Histamine in Tissue Growth and Repair

It has been suggested (*Asboe-Hansen, Dyrbye, Moltke & Wegelius 1959*) that tissue edema initiates any process of growth, regeneration and repair of connective tissue. As histamine induces increase of vascular permeability, thus producing a tissue edema, histamine release may be part of the very first response of the tissues to injury, and, thereby, an important stimulus of repair (*Asboe-Hansen 1963 a*). Besides inducing fresh edema, histamine may stimulate active transport processes across endothelial cytoplasmic barriers (*Alksne 1959*). Such a stimulation may also be important in growth and repair. *Kahlson (1962)* associates the general processes of growth and repair with high histamine-forming capacity.

Kahlson, Rosengren, Westling & White (1958), whilst studying the urinary excretion of histamine in the pregnant rat, found removal of the fetuses to result in a rapid return of the urinary histamine levels to control values. Rat fetal tissues were found to possess high histamine-forming capacity. Later however *Kameswaran & West (1962)* examined fetuses of various mammals, and found no consistent relationship between fetal growth and histamine-forming capacity. The cat and the ferret formed no histamine in fetal tissue, and the rabbit and the hamster only small amounts, while the rat and the guinea-pig had a high fetal histamine-forming capacity. Thus, again we have to deal with great species differences. The rat and the guinea-pig, which, among the fetuses examined, had by far the greatest ability to form histamine, have also an increased diamine oxidase (histaminase) concentration in the plasma, lymph and placenta in pregnancy (*Wicksell 1949, Carlsten 1950, Swanberg 1950*).

It has also been known for some time that diamine oxidase rises during human pregnancy (*Kapeller-Adler 1944, Anrep, Barsoum & Ibrahim 1947*). The function may be a protection of the maternal organism against histaminic effects (*McElin & Horton 1949*), possibly of fetal histamine (*Kahlson 1962*). The human fetus produces histamine (*Kahlson, Rosengren & White 1958*), and high histamine-forming capacity has been demonstrated in vitro in a variety of human fetal tissues including skin of fetuses aged 19 to

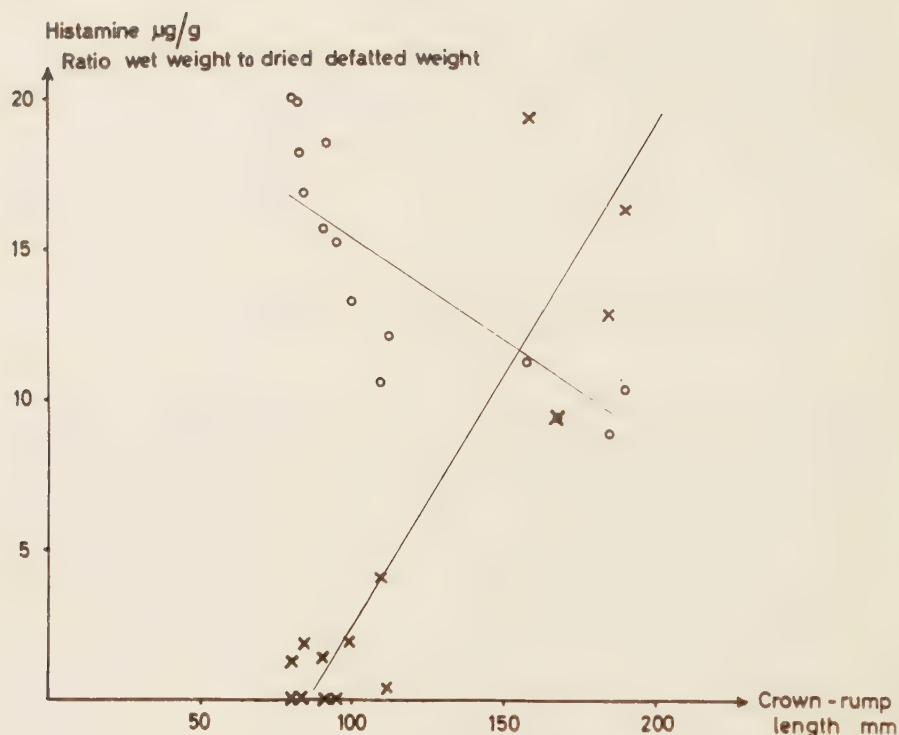


Fig. 9.

Histamine values (x) and ratio wet weight to dried defatted weight (o) of human fetal skin in relation to fetal age. Histamine values are expressed in μg histamine base per g dried defatted skin. Crown-rump values from 81 to 190 mm correspond to fetal ages from 12 to 20 weeks.

24 weeks (Lindberg, Lindell & Westling 1963). Investigations by Zachariae (1964 g) revealed a rise in skin histamine with increasing fetal age. Histamine and mast cells did not appear in human skin in larger quantities until the fifth fetal month (fig. 11). The investigations also demonstrated a high ratio of wet weight to dry defatted weight, declining with increasing fetal age (fig. 11). This is in agreement with the suggestion that tissue edema invariably initiates processes of connective-tissue growth. From the results of the investigation it was proposed that, in early human fetal life characterized by rapid growth, the demand for histamine is so considerable that a storage of cell bound histamine in skin can scarcely take place.

Marckmann & Zachariae (1964, 1965) studying histamine and mast cells in full thickness skin autografts of rats and pigs, found that a considerable release of histamine took place in the graft during the first days after operation (fig. 10 and 11). A decrease in the number of identifiable mast cells was also observed. The experiments on pigs were performed using a

Histamine per cent
of original value

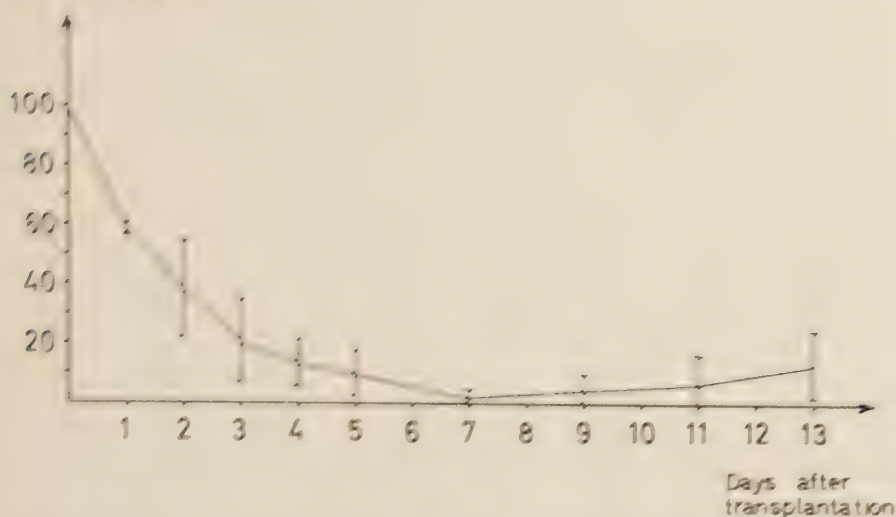


Fig. 10.

Average amount of histamine in full-thickness skin grafts of pig, expressed in per cent of original value, followed up to the thirteenth day after the operation. Standard deviations are shown with the average values.

histamine introduced by Clemmensen (1963). The decrease of the histamine values in the skin grafts was similar to that found by other workers in linear wounds (Kahlon, Nilsson, Rosengren & Zederfeldt 1966, Håkberg, Jørgensen, Schmidt & Iskov 1961). The microscopic studies of the grafts revealed an edema. Clemmensen (1963) in his study on the early circulation in full thickness skin grafts of pigs found an increase in the weight of the grafts on the second, third and fourth day after the operation, this also indicating an edema. In the rat (Marckmann & Zachariae 1964) the water content in the grafts was measured disclosing an increase. Histamine probably increases vascular permeability in the grafts and in the wound bed. Whether histamine release precedes and produces edema or the edema is primary and responsible for the release of histamine has not been established.

In the studies on full thickness autografts of pig, microscopy revealed a superficial necrosis to take place regularly. The necrosis was followed by a development of new epidermis from the epithelial cells of the hair sheaths. This was not the case in full thickness autografts of rat skin. Only one rat autograft out of forty seven showed signs of decreased vitality. Pig skin differs from rat skin by containing one main concentration of mast cells and histamine in the subepidermal layer of the dermis (see chapter III), whereas

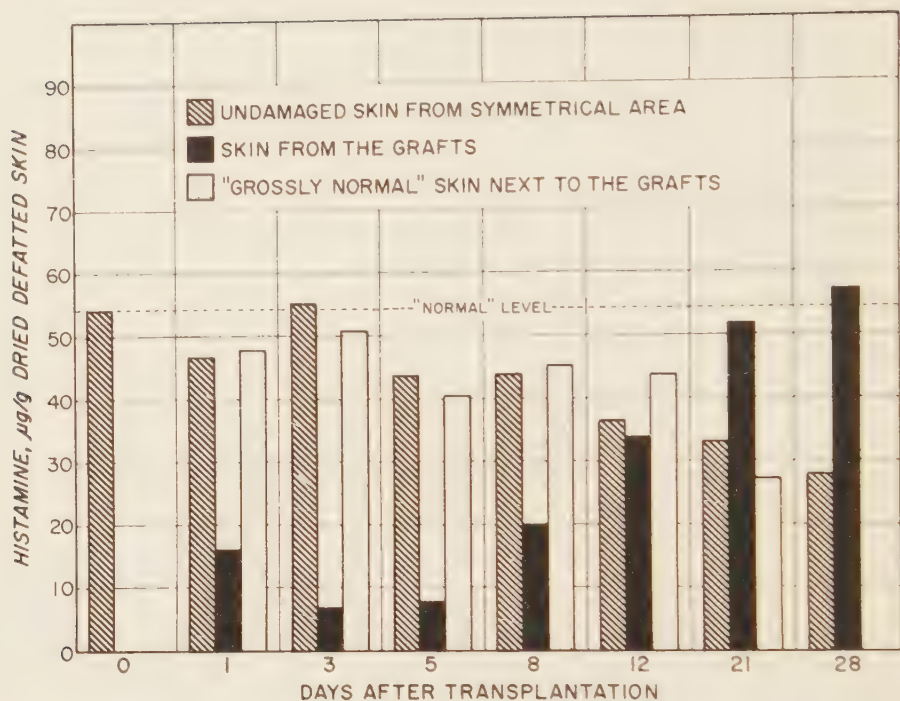


Fig. 11.

Average amounts of histamine in full thickness skin grafts of rat, in undamaged (uninjured) symmetrical skin, and in grossly normal skin next to the grafts, followed up to the twenty eighth day after the operation. The values are μg histamine base per g dried defatted tissue.

the mast cells and histamine in the rat are mainly located in an inner layer close to the base of the graft. This closer relation to the vessels of the wound bed may be of importance to the survival and the early repair in full thickness skin grafts of rat.

Zachariae (1964 f) also studied skin histamine in induced primary irritant dermatitis in man. The primary irritant reactions were produced with white spirit of petroleum in eighteen patients with contact dermatitis, and appeared as erythema and edema. After 48 hours skin histamine levels were investigated in irritated skin and control skin from symmetrical areas. Although an edema was detectable, no significant alterations in skin histamine could be found. The average skin histamine level of primary irritant reactions was $19.7 \mu\text{g}$ per g dried and defatted skin in comparison with $18.7 \mu\text{g}$ g for normal skin from symmetrical areas. The average increase in water content was 5.5 per cent, ranging from 1.3 to 11.0 per cent. The failure to demonstrate histamine alterations in the skin in this case does not, however, argue strongly against histamine as a mediating agent. The histamine levels

were only investigated 48 hours after the application of the irritant, and released histamine may already have been compensated by an increased histidine-decarboxylase activity. *Kahlson, Nilsson, Rosengren & Zederfeldt* (1960) found that rat wound tissue excised 24 hours after infliction of a wound had a high histamine-forming capacity. The histidine-decarboxylase level was 50 to 60 times the level in control skin. When the histamine-forming capacity was reduced by semicarbazide superimposed on a pyridoxine deficient diet, healing was greatly retarded.

Kahlson (1960) also investigated the histamine-forming capacity of granulation tissue from implanted plastic sponges in rats. Fresh granulation tissue resembles the connective-tissue of fetal skin. In the granulation tissue the histamine-forming capacity reached a high peak on the fifth day and then fell steeply. In the fresh granulation tissue of the pig *Marckmann & Zachariae* (1965) found no mast cells and no measurable histamine from the third to the fifteenth day after wounding. Also here, the demand for histamine may be so considerable that a building up of cell bound histamine can not take place.

When following histamine levels in the full thickness skin graft of the rat, after the fifth day *Marckmann & Zachariae* (1964) noticed a gradual increase (fig. 11). On the twenty first and twenty eighth day the histamine contents of the grafts had exceeded skin histamine from symmetrical areas and had reached the levels of the control animals. Skin histamine values of uninjured skin in the operated animals slowly decreased from the third to the twenty-eighth day, indicating that regeneration of skin histamine of the graft may take place at the expense of normal skin. No significant differences in skin histamine were found between uninjured skin from symmetrical areas and grossly normal skin next to the grafts.

Malignant growth is another type of rapid tissue growth. As far back as 1906 *Fromme* first claimed that the mast cells destroy the toxic substance of cancer. In a study of carcinoma of the uterus and mamma he noted that in deeply affected areas the number of mast cells was remarkably decreased, while in areas about to become cancerous mast cells became grouped in the periphery. Since then numerous investigators have asserted that mast cells display a role of defence against cancer and other tumours, the cells tending to accumulate in the immediate neighbourhood between the normal and abnormal tissue. *Weill* (1919) reported massive accumulation of mast cells surrounding sarcoma metastasis. *Cornil & Michon* (1924) encountered a large number of mast cells in subcutaneous tumours in six cases of von Recklinghausen's disease. *Higuchi* (1930) claimed that in 22 cases of carcinoma of the breast, the mast cells were extremely rare in the tumours, while in the neighbourhood between the normal and carcinomatous tissue the cells were abundant. In 1944, *Cramer & Simpson* were impressed by

the marked increase in mast cells around incipient neoplastic reactions produced experimentally with methylcholantrene. They suggested that mast cells may play some role in inhibiting neoplasia, because these cells were most numerous in tumour resistant strains of mice and because the mast cell count declined when the tumours became malignant. Their observations were confirmed by *Engelbreth-Holm & Asboe-Hansen* (1953) studying experimental skin tumours of mice.

The demonstration of histamine in mast cells has aroused new interest in this subject. During recent years several studies on experimental animals have been performed in attempts to elucidate the relationship between histamine, mast cells and tumour growth. These studies seem to indicate that some malignant tumours are partially dependent upon histamine for growth. *Mackay, Marshall & Riley* (1960) found a high rate of histamine formation in rats bearing a subcutaneously implanted hepatoma, the rate of histamine formation declining to normal on removal of the tumour. *Håkansson* (1961) studied transplants from the Walker rat mammary carcinoma. The histamine forming capacity was high in the cancer tissue and low in normal mammary tissue. The histamine content of both kinds of mammary tissue was low, and mast cells were not found in the tumour tissue. In the Landschütz I tumour growing in the mouse peritoneal cavity as single free cells *Kahlson, Rosengren & Steinhardt* (1961) found that, on the first day of tumour growth, the histidine-decarboxylase activity was spectacularly elevated and correlated with the frequency of tumour cell mitosis. *Scott* (1963) showed that, when histamine and 5-hydroxytryptamine were given one day prior to tumour implantation and daily until the tumours were removed after seven days, the tumours were significantly larger than the controls. He also studied the effect of histamine and 5-hydroxytryptamine antagonists on rat sarcoma implants and found that histamine and 5-hydroxytryptamine blockers possess moderate anti-cancer activity. As mentioned before (chapter IV), rat and mice mast cells besides histamine contain 5-hydroxytryptamine. *L. Zachariae & Asboe-Hansen* (1958) found that hydrocortisone acetate, when injected topically into skin carcinomas of mice, may result in a temporary regression of the tumours. Corticosteroids are believed to inhibit histamine biogenesis (see chapter XI) as well as other metabolic activities.

In a limited number of mice with experimental skin tumours induced by painting the skin with 9,10-dimethyl-1,2-benzanthracene the present author intended to study the possible influence of the histamine liberator polymyxin B (see later, Chapter XI). In this study, the histamine determinations of the tumours showed that the skin carcinomas of both groups, treated as well as untreated animals, had a lower average histamine value than the non malignant skin papillomas ($p < 0.0025$), even when excluding the very

high histamine value of one non malignant skin papilloma (case nr. 13 from table 13). The average value of four carcinomas was found to be 67.5 ± 33.3 $\mu\text{g/g}$, the values ranging from 26.6 $\mu\text{g/g}$ to 166.7 $\mu\text{g/g}$ (table 15), while the non-malignant papillomas, excluding case nr. 13, showed an average histamine value of 271.2 ± 43.6 $\mu\text{g/g}$ with a range of values from 60.7 to 625.0 $\mu\text{g/g}$. The average value of a third group of papillomas sus-

Table 13
Characteristics of non-malignant skin papillomas

Case no.	Wet weight of tumour (mg)	Treatment	μg histamine per g dried, defatted tumour	Estimated quantity of mast cells*)
1.....	22.5	Polymyxin B.....	330.3	++
2.....	48.4	".....	473.5	++
3.....	13.1	".....	60.7	++
4.....	53.6	".....	101.2	++
5.....	22.5	".....	343.7	++
6.....	39.0	".....	492.4	++
7.....	47.2	".....	342.4	+++
8.....	37.7	".....	79.5	++
9.....	94.5	".....	161.2	++
10.....	43.9	".....	108.7	++
11.....	15.2	".....	114.8	†)
12.....	30.5	Physiologic saline..	98.7	++
13.....	55.7	".....	3009.4	++
14.....	34.5	".....	230.0	†)
15.....	14.9	".....	410.7	+++
16.....	12.1	".....	625.0	†)
17.....	22.3	".....	365.9	+++
Mean (excluding case no. 13).....			271.2	
Standard deviation.....			± 174.4	
Standard deviation of the mean.....			± 43.6	

*) See table 15.

Table 14
Characteristics of skin papillomas suspected for beginning malignancy

Case no.	Wet weight of tumour (mg)	Treatment	μg histamine per g dried, defatted tumour	Estimated quantity of mast cells*)
1.....	25.7	Polymyxin B.....	388.4	+
2.....	108.3	".....	101.3	+
3.....	92.0	".....	96.4	+
4.....	82.0	Physiologic saline..	364.8	†)
Mean.....			237.7	
Standard deviation.....			± 160.7	
Standard deviation of the mean.....			± 80.4	

*) See table 15.

Table 15
Characteristics of skin carcinomas

Case no.	Wet weight of tumour (mg)	Treatment	μg histamine per g dried and defatted tumour tissue	Estimated quantity of mast cells*)
1.....	45.4	Polymyxin B.....	166.7	+
2.....	1641.0	“.....	44.9	0
3.....	152.5	Physiologic saline..	26.6	(+)
4.....	135.0	“..	31.7	0
Mean.....			67.5	
Standard deviation.....			± 66.6	
Standard deviation of the mean.....			± 33.3	

*) The quantity of mast cells in the sections of the tumours selected for the microscopic examination are described with the figures: 0, (+), +, ++, and +++ where 0 means no mast cells, (+) very few mast cells, + normal quantity of mast cells, ++ an increased amount and +++ a greatly increased amount of mast cells. The figure † indicates the sections too small to allow any estimation of the quantity of mast cells.

pected for beginning malignancy was $237.7 \pm 80.4 \mu\text{g/g}$ with a range of 96.4 to $388.4 \mu\text{g/g}$ (table 14).

The microscopic studies also revealed a rough parallelism between the mast cell content and the histamine values of the different tumour groups. The skin carcinomas showed almost no mast cells in their connective tissue, while the non-malignant papillomas were rich in mast cells. The group of skin papillomas which were suspected of beginning malignancy showed a varying content from few to a moderately increased number of mast cells. All this information should be taken with reservation, as the material selected for the microscopic examination was scarce, and the number of animals included in this experiment small. The disappearance of the mast cells with the increasing malignancy of the skin tumours, however, agrees with declining histamine values, and leads to the speculation as to whether it is of advantage to the host or to the tumour. As mentioned above, *Cramer & Simpson* (1944) suggested that mast cells inhibit neoplasia, while more recent studies seem to indicate that malignant growth, as maybe all rapid tissue growth, requires histamine.

A likely explanation might be that the demand for mast cell constituents, among these histamine in malignant growth is so considerable that, when a tumour turns malignant, mast cells in the connective tissue of the neoplasia release their granules and, thereby, lose their microscopical characteristics. As the demand for mast-cell constituents, among these histamine, may be expected to be continuous, the possibility exists that, although hyperactive, the cells do not regranulate. Further studies are needed concerning this matter.

Considering the probability of an influence of histamine on tissue repair

and growth, non-malignant as well as malignant, it is concluded, that tissue edema initiates the processes and that histamine may play a part increasing vascular permeability. The disappearance of mast cells suggests that there is a connection between tissue repair and the mast cell. Conclusive evidence for this hypothesis, however, is lacking, and the findings may only be reflections of secondary changes occurring in the connective tissue.

Schayer (1963) suggests that newly formed "induced histamine" is instrumental in the control of the microcirculation in damaged regions. According to *Kahlson* (1960) histamine, as linked with physiological events is not formed from mast cells. These workers, however, have not been able to state the location of neither "induced histamine" nor "nascent histamine", the term introduced by *Kahlson* (1962), implying increased formation of histamine in rapidly growing tissues. Mast cells at present are only identifiable when being granulated. And there exists no direct evidence that these forms of histamine are not associated with degranulated mast cells, immature mast cells, or precursors not yet identifiable as mast cells.

Chapter VI

Skin Histamine and Immediate Allergic Reactions

One of the first recorded instances of what must have been an immediate allergic reaction in animals was reported by *Magendie* in 1839, who discovered that death ensued after a repetition of the injection of egg white in a dog that had already been injected with that substance. In 1902 *Portier & Richet* concluded that one inoculation of a given protein tends to render an animal hypersensitive to that protein, and, on the basis of this observation, they coined the term "anaphylaxis". *Arthus*, in 1903, observed that rabbits inoculated subcutaneously for the second time with the same serum, develop a local area of inflammation and necrosis at the point of injection. This resulted from previously established hypersensitiveness. *Von Pirquet*, in 1906, employed the term allergy to denote a similar kind of sensitivity in human beings.

The immediate-type of response is mediated by circulating antibodies. Anaphylaxis is an immediate type reaction which may be induced experimentally in animals, and occasionally in man, by exposure, usually through injection for the second time, to the same antigen. It results from a specific union of antigen and antibody in tissues, as a result of the fixation of one of the reactants to the tissue. Anaphylaxis is manifested by a violent, often fatal reaction. The Arthus' phenomenon and serum sickness are two other forms of induced immediate reactions (see *Criep* 1962, see *Bruun & Hagerman* 1964). The Arthus' phenomenon is a local type of allergic reaction, that develops after repeated subcutaneous or intradermal injections of an antigen. It differs from the anaphylactic reaction in that no fixation of antigen or antibody seems necessary to produce it, therefore no latent period is needed. According to *Criep* (1962), humoral substances play no part in producing the lesions. The damage is supposed to be due to intravascular thrombosis.

Besides induced immediate allergic reactions we have "familial" spontaneous reactions, "atopy", the skin manifestations being urticaria, angio-neurotic edema and atopic dermatitis (prurigo Besnier) (see *Criep* 1962,

see *Bruun & Hagerman* 1964). No definite proof, however, has been presented of antigen-antibody reactions being the cause of atopic dermatitis, and urticaria and angioneurotic edema may both be produced by a great variety of substances unassociated with antigen-antibody reactions.

Between the discovery of histamine in 1907 by *Windaus & Vogt* and the demonstration in 1932 by *Dragstedt & Gebauer-Fuelnegg* of histamine release from the tissue of the sensitized animal on contact with an antigen, the concept of the participation of histamine in anaphylactic reactions was mainly based on the analogies between its pharmacological effects and those typical of anaphylaxis. This analogy was stressed by *Dale & Laidlaw* (1910), *Dale* (1913) and *Lewis* (1927). Since then, several investigators have published experimental work on histamine and the immediate allergic reactions of the skin (*Bartosch, Feldberg & Nagel* 1932, *Feldberg & Schachter* 1952, *Schachter* 1953, *Inderbitzin* 1955, *Inderbitzin* 1961) giving evidence of histamine release in connection to cutaneous anaphylaxis in the rat, guinea-pig, dog, cat and rabbit.

Inderbitzin (1955) also studied skin histamine in passive cutaneous anaphylaxis and the reversed passive Arthus reaction in rabbits. In the passive cutaneous anaphylaxis reaction, the injection of antigen was followed by a rapid decrease in skin histamine content at the site of the reaction. Within twenty minutes the content fell to 50 per cent of that of the control skin. In the reversed passive Arthus reaction a large amount of antigen was injected intraperitoneally. After 60 to 90 minutes 0.2 ml rabbit antiserum (antibody) in saline was injected intracutaneously. In this case, after an initial rise, due possibly to histamine in the injected solution, the histamine content of the skin at the site of the reaction fell to about 30 per cent of that of the control area. After six hours, the loss, however, was followed by an increase which sometimes exceeded 200 per cent. This histamine increase coincided roughly with an infiltration consisting of polymorphonuclear and non-classified mononuclear cells. *Inderbitzin*, in 1955, associated the secondary histamine rise with the polymorphonuclear leukocytic infiltration. According to our knowledge of to-day (see later, chapter IX), probably the rise must be attributed to basophils.

On the basis of experiments made on isolated organs of the guinea-pig *Dale* (1929) concluded that anaphylaxis is a cellular phenomenon. *Uvnäs* and co-workers have studied the mechanisms of histamine release from isolated rat mast cells (*Högberg & Uvnäs* 1957 and 1960, *Diamant & Uvnäs* 1961, see also *Uvnäs* 1963), following antigen-antibody reactions as well as administration of histamine liberators. In both cases morphological changes were found in the cells. The influence of pH, ionic milieu, temperature, and enzyme inhibitors indicated to the authors a participation of enzymatic

mechanisms, while the influence of metabolic inhibitors, anoxia, and glucose protection suggested that energy is required.

Sensitized mast cells may be damaged by antigen when an anaphylactic release of histamine is produced. However, views diverge regarding the importance of histamine in anaphylaxis and other types of immediate allergic reactions. Lately, the interest has also been focused on other substances, among these especially 5-hydroxytryptamine (serotonin) (*Humphrey & Jaques* 1953) and "slow reacting substance" (*Kellaway & Trethewie* 1940). A release of both of these substances may take place parallel to histamine release (*Chakravarty* 1960, *Garcia-Arocha* 1961, *Moran, Uvnäs & Westerholm* 1962). Besides we may record that the contraction of isolated actinomyosin fibers has been demonstrated under circumstances where humoral factors have not been able to participate (*Lehotan & Hasik* 1959).

Evidently, there is considerable species differences in skin allergies. Although histamine is released from the skin in immediate allergic reactions both in the rat and the guinea-pig, while it may play a major role in anaphylaxis in the guinea-pig, it seems to play only a minor role in the rat and the mouse. Rats and mice seem special among the species in having demonstrable quantities of 5-hydroxytryptamine along with histamine in their mast cells. It was suggested by *Benditt, Holcenberg & Lagunoff* (1963) that in the two groups of rodents the functions of histamine have been in a large part pre-empted by 5-hydroxytryptamine. *Brocklehurst, Humphrey & Perry* (1955 and 1960), however, observed that rats depleted of skin histamine and 5-hydroxytryptamine and pretreated with histamine and 5-hydroxytryptamine antagonists still showed a strongly positive passive cutaneous anaphylaxis. Furthermore *Mota* (1963), studying mast cells and anaphylaxis, reports that the experimental data so far obtained have shown that mast cell-damage by antigen invariably occurs under circumstances in which an anaphylactic release of histamine is produced in actively sensitized guinea-pigs, rats and mice, but never has been observed in anaphylaxis of passively sensitized rats and mice. He suggests that mast cells play a very important role in guinea-pig anaphylaxis due to a high sensitivity of this species to histamine, but probably an insignificant role in rat due to a low sensitivity of this species to histamine and 5-hydroxytryptamine. Nevertheless, in both species the reaction of antigen with antibodies fixed on the mast cells will induce damage to these cells which, by a yet more or less unknown mechanism, causes histamine liberation.

In man, whose skin has a relatively high sensitivity to histamine, both mast cell degranulation (*Asboe-Hansen* 1950 a) and histamine release (*Zachariae* 1963 c) have been demonstrated in urticaria, a condition which, in certain instances may be considered an immediate allergic reaction of

the skin (see chapter VIII). As mentioned before human mast cells apparently do not contain 5-hydroxytryptamine.

It is concluded from evidence which is by no means as complete as we would like it to be, that histamine release is found to take place in allergic immediate reactions of the skin, but that it may not in all cases be recognized as the pharmacologically active substance responsible for the clinical manifestations.

Chapter VII

Histamine and Delayed Skin Reactions

The delayed types of allergy (cellular sensitivity, cytoallergy) are reactions of hypersensitivity not associated with circulating antibody. They may be non-infectious or infectious. The allergic contact dermatitis belongs to the first group, the tuberculin type reaction to the latter (see *Criep* 1962, see *Bruun & Hagerman* 1964).

While half a century has elapsed since *Dale & Laidlaw* (1910) suggested the participation of histamine or a histamine-like substance in immediate allergic reactions, it is only within recent years, that the first reports on alterations in skin histamine of the delayed type of hypersensitivity were published (*Inderbitzin* 1955, *Fischer & Cooke* 1958). This research disclosed a steady rise in delayed reactions, typified by the experimental dinitrochlorobenzene contact dermatitis and the tuberculin reaction. The rise reached its maximum 24 to 72 hours after the injection or application of the sensitizing agent.

Zachariae (1964 f) studied skin histamine in experimental allergic contact dermatitis as well as in induced primary irritant dermatitis in human patients suffering from allergic contact dermatitis. No significant alterations were found in primary irritant reactions or in the initial stages of allergic reactions. After 24 and 48 hours, however, a marked rise in skin histamine was observed in the positive allergic patch tests (fig. 12).

The process of the local histamine accumulation in allergic patch tests coincides with a dermal immigration of basophil leukocytes demonstrated in human experimental contact allergy by *Wolf-Jürgensen* (1962) and *Aspegren, Fregert & Rorsman* (1963 a). This correlation may explain the local increase in histamine, which previously was attributed to lymphocytes (*Inderbitzin* 1961). The absence of a similar histamine rise in the primary irritant reactions agrees with the results of *Aspegren et al.*, who found no metachromatic cells in the vesicles of the skin treated with irritants.

Although histamine accumulation may also be found in various non-allergic inflammatory skin reactions (*Craps & Inderbitzin* 1957), the investigations of *Zachariae* (1964 f) indicate that, in patch tests, a significantly

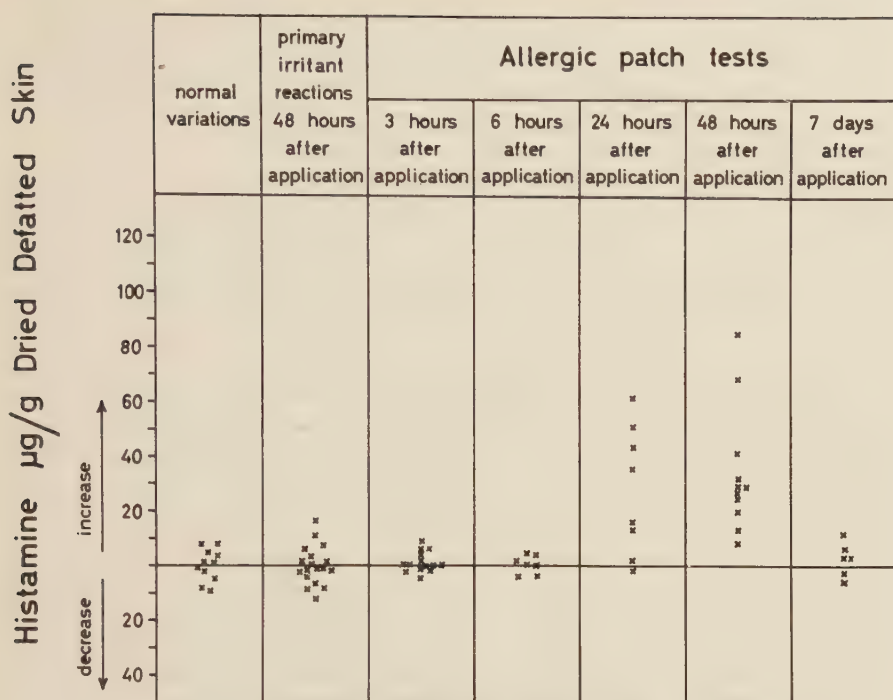


Fig. 12.

Alterations in skin histamine in allergic patch tests and primary irritant reactions compared with normal variations in skin histamine from the same area.

higher histamine level is associated with allergic response in contrast to non-allergic reactions. This observation has bearing upon the differential diagnosis between allergic and primary irritant skin reactions in patch tests.

Concerning the function of the accumulated histamine, we know from the research of *Uvnäs* and co-workers (see *Uvnäs* 1963) that histamine release may take place from isolated mast cells following antigen-antibody reactions. Blood basophils are considered to belong to the mast cell system (see *Boseila* 1959) and to represent a mobile source of histamine (*Graham, Lowry, Wheelwright, Lenz & Parish* 1955). In the delayed type of skin allergy the immigration of basophils from the blood stream into the skin may contribute to the source of releasable histamine, already present in the tissue mast cell. Thus the antigen-antibody reaction may lead to a continuous liberation of histamine contributing to long lasting increased vascular permeability and edema.

In favour of this hypothesis we may recall the reports of *Mayer* (1947) and *Lecomte* (1952), who were able to diminish edema formation in eczematous or tuberculin reacting animals with local application of anti-

histaminic compounds. *Nilzén* (1950) found that histamine antagonists, if added to the test solutions in patch tests, regularly exerted an inhibitory influence upon the patch-test response. The ineffectivity of antihistaminics in clinical use in allergic contact dermatitis probably may be due to insufficient amounts at the site of action.

As to the initial stage of the delayed allergic reaction, no significant differences in skin histamine have been found (*Inderbitzin* 1955, *Fischer & Cooke* 1958, *Zachariae* 1964 f). But this does not argue strongly against histamine as a mediating agent. The processes of release may take place so slowly, that released histamine is compensated by histamine in the basophil leukocytes.

This, however, is mere speculation. Although experimental results suggest that histamine may have a part to play in delayed allergic reactions of the skin, until now nothing indicates that histamine is essential for the onset of the vascular disturbances in this type of allergy.

Chapter VIII

Skin Histamine in Itching Dermatoses

1. Atopic dermatitis

Atopy is supposed to be a form of familial allergy associated with circulating antibody. The term "atopy" was coined by Coca 1923. It means a strange disease. According to Crieep (1962), it is applied to clinical allergic conditions in man which are immediate, explosive, edematous, reversible and familial. It is associated with an immediate wealing type of reaction, and with blood and tissue eosinophilia.

Hypersensitivity is considered a conspicuous characteristic of individuals with atopic dermatitis (prurigo Besnier, neurodermatitis disseminata) with many patients upon skin testing revealing numerous "sensitivities". Experience, however, has been disappointing with regard to the elimination from the diet and environment of materials giving positive reactions to tests; and desensitization procedures based on skin testing have been abandoned by most experienced dermatologists because of consistently poor results (*Sternberg & Newcomer* 1959). This, together with the fact that no definite proof has been presented of antigen-antibody reactions being the cause of the skin symptoms of atopic dermatitis, is the reason for placing the disease under the more neutral heading of itching dermatoses. Besides, pruritus which accompanies atopic dermatitis undoubtedly is the most difficult of the symptoms to manage.

The histological picture usually is that of a chronic dermatitis showing acanthosis with varying degrees of spongiosis. Eosinophils are often present, but usually their number is small (*Lever* 1961). In the corium considerable amounts of acid mucopolysaccharide and a large number of mast cells have been found (*Asboe-Hansen* 1951).

The skin histamine levels in chronic atopic dermatitis have been investigated spectrophotometrically by *Johnson, DeOro, Lascheid & Mitchell* (1960), who examined skin from the antecubital fossae of fifteen normal adults and from sixteen patients with adult atopic dermatitis. They found normal values ranging from 4.2 to 14.3 $\mu\text{g/g}$ (wet weight) with an average of 9.2 $\mu\text{g/g}$, while involved skin from the patients with atopic dermatitis had

an average skin histamine level of 16.0 $\mu\text{g/g}$ (wet weight) with a range of 9.7 to 24.8 $\mu\text{g/g}$, thus indicating an increased skin histamine level in atopic dermatitis. Also Nilzén (1958) using bio-assay has been able to demonstrate relatively high skin histamine values in atopic dermatitis.

2. Urticaria

Urticaria, a vascular reaction pattern of the skin, characterized by transient appearance of weals that are localized areas of edema, was for long regarded as a symptom of antigen-antibody reaction. Recent attention, however, has been focused on a large group of substances found capable of acting as histamine liberators (see chapter XI), these substances produce urticaria. In addition to this, there are stimuli such as heat, exercise and psychogenic factors, all unassociated with immunologic response, which can produce urticaria. Therefore we prefer to place also urticaria among itching dermatoses.

According to Lewis & Grant (1924) the appearance of weals in the skin may be the result of the liberation of a histamine-like substance. Evidence in support of histamine liberation in urticaria has therefore been sought in various ways: by observations on the urinary excretion of histamine (Adam, Hunter & Kinnear 1950), by quantitative estimation of histamine in blood (Rose 1941, Rorsman & Rosengren 1958), by the action of anti-histamine drugs (Feinberg 1946, Hunter 1947, Bovet & Bovet-Nitti 1948), and by the extraction of histamine from the skin. Apart from the study of local reaction, it has been shown that systemic effects, comparable with those of histamine, may occur in urticaria (Horton & Brown 1929).

Several workers have studied skin histamine in urticaria (Pellerat & Murat 1946, Nilzén 1947, Meneghini & Levi 1958, Zachariae 1963 c). In the studies of Zachariae (1963 c) in no case the histamine content of the diseased skin was higher than of the corresponding grossly normal skin, and the average value of histamine in the weal was markedly lower than the average value in grossly normal skin, thus indicating that histamine is liberated from the skin in urticaria. This agrees with the earlier studies by Nilzén (1947) and the work of Pellerat & Murat (1946); while it is contradictory to the results of Meneghini & Levi (1958), who found higher histamine levels in the diseased skin than in grossly normal skin of the same patients. The difference in results may be due to a different clinical phase. In the investigations by Zachariae (1963 c), the specimens of diseased skin were from weals not more than four hours old, and the majority less than two hours old, while no definite age of the lesions have been stated by Meneghini & Levi. In early weals, i. e., those only few minutes old, one finds either no inflammatory reaction or merely a slight infiltrate composed of lympho-

cytes, while weals an hour or more old show a mild or even moderately severe cellular infiltrate around the vessels (*Török & Lehner 1921*). *Inderbitzin* (1961¹) in a work on the effect of acute and delayed cutaneous allergic reactions on the amount of histamine in guinea-pig and rat skin found that in immediate allergic reactions an initial rapid fall was succeeded by a moderate rise. As mentioned before urticaria resembles and in some

Table 16

Skin histamine levels in grossly normal, dried and defatted back skin from patients with spontaneous urticaria

Case no.	Sex	Age	Skin histamine ($\mu\text{g/g}$)
1.....	F	14	26.3
2.....	F	18	15.7
3.....	M	18	24.5
4.....	M	20	12.2
5.....	F	21	17.5
6.....	M	17	39.5
7.....	M	17	31.2
8.....	M	23	26.5
9.....	F	24	14.4
10.....	M	29	26.3
11.....	M	29	20.0
12.....	F	31	11.0
13.....	F	31	43.4
14.....	F	31	27.5
15.....	M	32	45.8
16.....	M	33	10.9
17.....	F	34	21.0
18.....	M	38	18.5
19.....	F	46	12.4
20.....	M	47	30.1
21.....	F	48	36.1
22.....	F	48	23.0
23.....	F	49	33.2
24.....	F	49	18.1
25.....	F	49	16.8
26.....	M	43	25.7
27.....	F	52	12.7
28.....	M	51	25.8
29.....	M	52	18.8
30.....	M	58	25.8
31.....	F	59	46.5
32.....	F	64	28.4
33.....	F	67	27.8
34.....	M	69	28.4
Mean.....			24.8
Standard deviation.....			± 9.7
Standard deviation of the mean.....			± 1.7

Table 17

Skin histamine in wealing and grossly normal, dried and defatted back skin from a patient suffering from cold urticaria

Sex	Age	Skin histamine ($\mu\text{g/g}$)	
		Grossly normal skin	weal
F.....	21	17.5	8.1

cases may be due to an immediate allergic reaction. Meneghini and Levi may have taken their biopsies in the secondary phase, probably associated with infiltration of basophils. Basophils, although few, have in some cases been observed in urticarial weals (*Rorsman* 1961).

Histamine in grossly normal back skin from 34 adult patients with urticaria was investigated by the present author and found to average $24.8 \pm 1.7 \mu\text{g/g}$ with a range of 10.9 to $46.5 \mu\text{g/g}$ (dried defatted weight) (table 16). This is almost within the range of normal values for the area (from 11.7 to $42.6 \mu\text{g/g}$). The average value of the patients with urticaria differed only insignificantly from the average skin histamine level of back skin from normal individuals, which has been found to be $26.4 \mu\text{g/g} \pm 4.2 \mu\text{g/g}$. The investigation included both patients with acute and chronic urticaria. Concerning the treatment of urticaria see later (chapter IX).

Table 18

Skin histamine in wealing and grossly normal, dried and defatted back skin from patients suffering from urticaria factitia. The specimens were removed 10 minutes after the weals were produced by scratching the back with a match

Case no.	Sex	Age	Skin histamine ($\mu\text{g/g}$)	
			Grossly normal skin	weal
1.....	F	18	26.3	21.0
2.....	M	20	42.9	31.4
3.....	M	20	15.9	12.8
4.....	M	32	45.8	31.2
5.....	M	32	24.6	15.5
6.....	M	36	41.4	35.3
7.....	F	48	12.1	11.6
Mean.....			29.9	22.7
Standard deviation.....			± 13.6	± 9.9
Standard deviation of the mean.....			± 5.1	± 3.7

Besides in spontaneous urticaria evidence of histamine release was also found in urticaria factitia (dermographic prurigo) and in a patient suffering from physical urticaria due to cold (table 17). In urticaria factitia the urticarial weal is produced traumatically. In order to produce weals in seven patients suffering from urticaria factitia, the skin of the back was scratched. The least force necessary was applied. Specimens of wealing skin were removed 10 minutes after the stimulation together with samples of grossly normal skin taken symmetrically for control. Here, as in spontaneous urticaria, the histamine content of wealing skin was lower than that of corresponding grossly normal skin (table 18). The average reduction in skin histamine in wealing skin was $7.2 \pm 1.9 \mu\text{g/g}$. The reduction was significant ($p < 0.005$). The results presented here correspond with earlier reports on this phenomenon by Nilzén (1947). The number of patients examined does not allow any conclusions as to the general level of skin histamine in this condition. All determinations, except one, however, are within normal values for the area.

3. *Erythema multiforme and bullous pemphigoid*

In erythema multiforme, an acute, itching self-limited dermatosis, the most common lesion is the papule, which by peripheral extension and central clearing, tends to form an iris. When severe, erythema multiforme, as urticaria, may be bullous. Severe erythema multiforme, starting abruptly with high fever and an extensive, predominantly bullous eruption of the skin and the mucous membranes, usually is named Stevens-Johnson's syndrome. Lever (1957) regards bullous pemphigoid as a chronic variant of bullous erythema multiforme, while others believe it to be a bullous variant of dermatitis herpetiformis. Commonly, however, bullous pemphigoid is considered belonging to the bullous diseases of the pemphigus group.

The etiology of erythema multiforme, although a subject of speculation for years, is still obscure. Bacteria, virus, drugs and allergy have been suggested. The latter is probably the more generally accepted at present (see Andrews & Domonkos 1963).

In erythema multiforme, the epidermis may show spongiosis and intra-cellular edema, occasionally leading to intra-epidermal spongiotic blisters (Piérard, Dupont & Fontaine 1957). The dermis contains an inflammatory perivascular infiltrate, which is composed mainly of lymphocytes, but may contain neutrophils and eosinophils (see Lever 1961). Recently also basophils have been reported to accumulate in erythema multiforme lesions (Aspegren, Fregert & Rorsman 1963 b). As mentioned above, basophils hold a large content of histamine, while eosinophils may possibly be regarded as wandering cells capable of inactivating histamine (see chapter IX). Electron

Table 19

Skin histamine in grossly normal and diseased, dried and defatted skin from three patients with bullous pemphigoid

Case no.	Age	Sex	Skin area	Skin histamine ($\mu\text{g/g}$)	
				grossly normal skin	diseased skin
1.....	74	F	thigh.....	22.0	18.9
2.....	75	M	chest.....	32.6	27.0
3.....	83	F	upper arm...	23.1	94.0

Table 20

Skin histamine levels in grossly normal, dried and defatted skin from patients with erythema multiforme exudativum and bullous pemphigoid compared with average histamine values of normal human skin

Case no.	Sex	Age	Diagnosis	Skin region	Skin histamine ($\mu\text{g/g}$)	
					grossly normal skin of patient	average value of normal skin
1.....	M	24	Erythema multiforme exudativum	back	38.7	26.4
2.....	F	39	"	"	15.4	26.4
3.....	M	79	Bullous pemphigoid	"	20.7	26.4
4.....	M	74	"	chest	32.6	29.7
5.....	F	83	"	upper arm	23.1	22.3
6.....	F	26	Erythema multiforme exudativum	"	24.2	22.3
7.....	M	64	"	lower arm	23.8	30.7
8.....	F	63	"	thumb	23.3	25.5*)
9.....	M	22	"	thigh	30.4	27.0
10.....	M	26	"	"	29.3	27.0
11.....	M	38	"	"	40.1	27.0
12.....	F	74	Bullous pemphigoid	"	22.0	27.0
13.....	M	18	Erythema multiforme exudativum	lower leg	12.2	30.1
14.....	F	25	"	"	6.9	30.1
15.....	M	30	"	foot	16.2	29.0

*) Average value of normal skin from hand (dorsum).

microscopy has suggested that the primary lesion of erythema multiforme is in the dermis (Caufield & Wilgram 1962). The picture resembles that observed by Mājno & Palade (1961) in their studies on the effect of histamine and 5-hydroxytryptamine on vascular permeability. It may be added that erythema multiforme has been successfully treated with cyproheptadine (Periactin®), an antihistamine-antiserotonin drug (Asboe-Hansen 1963 b).

Zachariae (1964 c) studied the contents of histamine in diseased and grossly normal skin of ten patients with erythema multiforme exudativum. In no case the histamine content in the pathological skin was lower than in the corresponding grossly normal skin. It was suggested that the marked rise in skin histamine, together with the results of histologic and electron microscopic studies by other workers, and the results of clinical treatment with antihistamine drugs indicate that histamine may play a part in the pathogenesis of this disease.

The author also studied the contents of histamine in diseased and grossly normal skin of three patients with bullous pemphigoid (table 19). Here the number of patients studied is too small to allow any conclusions. It may be noted, however, that in two of the cases with bullous pemphigoid, there is no rise in the histamine content of the diseased skin.

Histamine in grossly normal skin from 15 adult patients with erythema multiforme exudativum and bullous pemphigoid was also investigated and found within the range of normal values for the skin areas involved (table 20).

4. Some general observations regarding histamine and itching

The possibility that histamine may be a general physiological stimulus for itching has been envisaged by Schachter (1952), Broadbent (1953) and Cormia & Kuykendall (1954). Schachter (1952) tested whether bile salts release histamine because of the phenomenon of pruritus in jaundice, and he found that they do so. Broadbent (1953) examined the mechanism of cowhage, or itching powder, the name given to the trichomes, which cover the pod of the tropical plant *Mucuna pruriens*. His results suggested that it contained a histamine liberator. It has subsequently been shown that the hairs of cowhage contain 5-hydroxytryptamine (Bowden, Brown & Batty 1954) and mucunain, a proteinase (Shelley & Arthur 1958). Broadbent (1953) converted glass wool into an effective itching powder when treated with a histamine liberator. Cormia & Kuykendall (1954) used histamine as a direct stimulus for itching. They found that the itch threshold was greatly lowered at night and in areas of dermatitic skin. Commonly dermatological diseases connected with itching are characterized by a rather high mast cell count in the corium and the connective tissue dominated by edema

(*Asboe-Hansen* 1951). As mentioned in this chapter, histamine seems to play a part in some itching dermatoses. Also in non-specified prurigo (*Meneghini & Levi* 1958) high histamine values have been reported found. *Feldberg* (1954) suggested that itching may be associated with histamine release in minute concentrations near the sensory nerve endings. But he admits that the whole problem is not clear. In the well known pruritus in man after morphine administration, for instance, in some cases the itching may be of peripheral and in other of central origin. This is based upon the observation that also intracisternally injected morphine in doses which do not allow sufficient amounts to reach general circulation produces itching. (See also *Arthur & Shelley* 1958).

Recently *Shelley & Arthur* (1958) have revealed an enzymatic basis for some types of itching. They isolated a proteinase, which they maintain is the active pruritogenic component of cowhage. They found that all endopeptidases, which are active at a pH of about 7, produce itching when introduced into the skin, even in surprisingly small quantities. As a result of this work they postulate that the endopeptidases are the chemomediators for pruritus and that these are released or activated in tissue as a result of trauma.

The conclusion is that although histamine when introduced into the skin produces itching, and it possibly plays a part in itching dermatoses, its role in symptomatic itching is still not elucidated.

Chapter IX

Histamine and Eosinophils

Erhlich (1879 a, b) coined the term eosinophils for leukocytes with granules showing an intense affinity for eosin. This property is generally used to identify and enumerate the cells. They originate in the bone marrow and by far the richest content is found there. In guinea-pigs this exceeds the peripheral blood content by about four hundred to one (*Hudson* 1960). The bone marrow, therefore, constitutes a great potential reserve of eosinophils. The mammalian blood eosinophils are relatively rare in comparison with the total leukocyte count. In human blood, as an approximate guide, a figure of from 50 to 300 per μl is probably normal (see *Hammarsten* 1957 and *Archer* 1963). Other figures, however, are also to be found in the literature. According to *Rud* (1947), eosinophils normally do not exceed 275 cells per μl , while in some textbooks an eosinophilia is not supposed to exist until the count exceeds 400 per μl (*Astrup*, *Brøchner-Mortensen & Faber* 1959, *Bruun & Hagerman* 1964), 450 per μl (*Brøchner-Mortensen* 1946), or 500 per μl (*Astrup & Jørgensen* 1963). *Uhrbrand* (1958) found the eosinophil count ranging from 40 to 600 per μl in 57 normal persons. The number of eosinophils tends to fall in the morning, whereas it rises in the afternoon (*Djavid* 1935, *Rud* 1947, *Uhrbrand* 1958).

Eosinophil levels or changes in eosinophil levels have been used to assay adrenal cortical hormones or ACTH (*Speirs & Meyer* 1949, *Rosenberg & Lewis* 1950, *Halpern* 1952, *Thorn*, *Forsham & Emerson* 1951, *Coste*, *Laurent & Delbarre* 1951, *Renold*, *Jenkins*, *Forsman & Thorn* 1952). They have been suggested to aid in the diagnosis of various clinical conditions such as Addisonian or Cushing's disease, Loefflers syndrome, parasitic infections, benzol poisoning, certain malignant tumours and allergy (*Bataglia* 1927, *Ringo* 1938, *Kirk* 1942, *Samter* 1949, *Löffler*, *Essellier*, *Demeyer & Morandi* 1952, *Best*, *Kark*, *Muehrcke & Samter* 1953, *Speirs* 1955, *Gross* 1959, *Criep* 1962, *Bruun & Hagerman* 1964). Eosinophils have been used as a means of detecting stress (*Heming*, *Sax & Holtkamp* 1952), of evaluating responses to surgical trauma (*Coppinger & Goldner* 1950, *Roche*, *Thorne & Hills* 1950) or dangerous amounts of radiation (*Moses & Platt* 1951), and have been found increased following the termination of most infectious diseases (*Ringo* 1938, *Kirk* 1942).

For many years it was postulated that eosinophils were the chief carriers of histamine in human blood (*Code* 1937, *Parrot, Gabe & Herrault* 1947). More recently, however, it has become evident that the blood basophil, despite its extreme scarcity, contains about fifty per cent of total blood histamine (*Graham, Lowry, Wheelwright, Lenz & Parish* 1955). As mentioned above, basophils are supposed to represent a mobile source of histamine (see *Boseila* 1959), whereas the stationary tissue mast cell represents most tissue histamine (*Riley & West* 1953). The similarity between these cells was stressed by *Erhlich*, who in 1891 named the basophil leukocytes "blood mast cells". During the last decade numerous reports have been published on the functional relationship of basophilic leukocytes and tissue mast cells (*Ehrlich* 1953, *Valentine, Lawrence, Pearce & Beck* 1955, *Asboe-Hansen & Kaalund-Jørgensen* 1956, *Boseila* 1958, *Lindell, Rorsman & Westling* 1961 b).

During recent years investigators have studied basophil-eosinophil relationship in human blood. *Hamerston, Elveback, Halberg & Gully* (1956) reported a statistically significant correlation between counts of both cell types in patients with convulsive disorders. *Code, Mitchell & Kennedy* (1954) found a parallel fall in the number of both cells after oral administration of cortisone, and *Osada* (1954) and *Boseila & Uhrbrand* (1958) found a reduction in the number of both cell types after injections of corticotropin (see also *Speirs* 1955).

Mast cells in connective tissues and eosinophils in blood and tissues also seem to be intimately related. *Riley* (1959) has reported a striking influx of eosinophils to tissues rich in mast cells when the latter are undergoing degranulation. Similar observations have been made by *Fernex* (1962). *Wulff, Zachariae & Øhlenschläger* (1964) studied the leukocytic response to aseptic inflammation in urticaria pigmentosa. As mentioned in chapter V, they found an extremely high percentage of eosinophils in early "skin window" preparation (fig. 7, page 35) (table 12, page 36). In two cases the eosinophils amounted to 40 to 50 per cent of the exudate cells, in the three remaining cases 10 to 20 per cent of the cells were eosinophils. Normally eosinophils in "skin window" preparations do not exceed 0.5 per cent of the total cell count (*Wolf-Jürgensen* 1962). The presence of basophilic granules was observed in many of the macrophages in all samples. It was suggested that the mechanical trauma made by scraping away the epidermis elicited mast-cell degranulation and histamine release and that the release of histamine caused emigration of eosinophils⁶⁾. It must be

⁶⁾ In later studies performed after the completion of this survey an emigration of eosinophils was found in skin windows following local injections of histamine or polymyxin B (*Wolf-Jürgensen & Zachariae* 1965). These studies were performed on normal skin of patients suffering from various dermatological diseases, urticaria pigmentosa not included. All patients had normal eosinophil counts in blood.

added that direct eosinophil counts were within normal ranges except in one patient, who showed a moderate increase (694 cells per μl).

As early as in 1932, histamine was reported to have a chemotactic effect upon eosinophils (*Kline, Cohen & Rudolph*). When injected into the skin a local eosinophilia appeared. This observation was confirmed by *Knott & Pearson* in 1934. According to *Archer* (1963) eosinophils might be regarded as wandering cells capable of inactivating histamine locally and rapidly in tissues on demand. The first report of an antihistaminic effect of eosinophils was published by *Kovács* in 1950. He stated that extracts of buffy-coat of blood rich in eosinophils antagonized histamine effects *in vitro*. *Vaughn* (1952, 1953) believed that eosinophils have a function in assimilating and destroying histamine. *Vercauteren* (1953) studied the properties of the isolated granules of horse eosinophils and found that extracts of these possessed an antihistaminic effect. *Archer, Feldberg & Kovács* (1962) demonstrated antihistaminic activity of suspensions of horse eosinophils and watery extracts of such suspensions. In more recent studies by *Archer* (1963) similar results were obtained with human eosinophils. Besides, it has been shown (*Archer* 1959) that intradermal histamine injections into skin rich in eosinophils is not accompanied by edema. *Archer* (1963) also reports that repeated injections of histamine at daily intervals into the same area of skin are followed only on the first occasion by edema formation, thereafter this effect is not seen. As, apart from eosinophilia, no histological abnormality was found, he concluded that the most obvious explanation of the absence of edema formation was an antihistaminic effect of the eosinophils.

Patients suffering from atopic dermatitis seem to have an increase of eosinophils in their circulating blood (see *Criep* 1962, see *Bruun & Hagerman* 1964), while this is normally not the case in urticaria (*Fink & Gay* 1934, *Rorsman & Rosengren* 1958).

Riis (1959) stated that local tissue eosinophilia is often associated with eosinophilia in the blood, but not always. Tissue eosinophilia according to *Lever* (1961) is prominent in, among other diseases, eczematous drug eruptions, atopic dermatitis and dermatitis herpetiformis. *Wolf-Jürgensen* (1962) besides basophils found an increased number of eosinophils in the exudate of the experimental dinitrochlorobenzene contact allergy. Also in urticaria, the perivascular cellular infiltration may show eosinophils (see *Criep* 1962).

One may speculate in a manner, which may explain both eosinophilia of blood or tissue and blood eosinopenia. Following histamine release the local concentration of free histamine in an organ is elevated, eosinophils from the regional blood will be attracted giving rise to a relative eosinopenia of the blood draining the area. If histamine release is massive, an eosinopenia in the differential count will follow immediately (*Archer* 1963). When

Table 21

Blood eosinophil counts before and prior to the last injection during treatment with 50 mg polymyxin B once weekly for 8 weeks, and clinical course judged one month after cessation of therapy

Case number	Age	Sex	Diagnosis*)	Leukocyte-count		Eosinophilia (Per cent of leukocytes in differential count and absolute count per μ l)		Clinical course after cessation of therapy
				Before treatment	Prior to last injection	Before treatment	Prior to last injection	
1..	35	F	U+A.E.	9500	10400	1% - 344 per μ l	7% - 675 per μ l	Improvement
2..	32	F	U	5900	6200	5% - 431 per μ l	6% - 794 per μ l	Improvement
3..	57	M	U	5700	6200	5% - 138 per μ l	18% - 1300 per μ l	No urticaria
4..	28	M	U.B.	6800	8600	2% - 69 per μ l	8% - 544 per μ l	Slight improvement
5..	27	F	U	6000	6900	3% - 175 per μ l	6% - 844 per μ l	Improvement
6..	29	M	U	4600	6000	5% - 163 per μ l	15% - 400 per μ l	No improvement
7..	52	F	U	6400	5400	2% - 213 per μ l	8% - 425 per μ l	Slight improvement
8..	43	M	U+A.E.	4200	4900	0% - 63 per μ l	3% - 419 per μ l	Improvement
9..	26	F	U+A.E.	7600	7500	1% - 169 per μ l	4% - 194 per μ l	Improvement
10..	21	F	C.U.	6200	11300	1% - 106 per μ l	9% - 756 per μ l	No improvement
11..	14	M	U+A.E.	4200	5000	8% - 269 per μ l	9% - 869 per μ l	Improvement
12..	17	M	U	5600	5700	1% - 94 per μ l	1% - 131 per μ l	Improvement
13..	46	F	U	4600	6000	1% - 138 per μ l	5% - 325 per μ l	No improvement
14..	32	M	U	11300	6200	5% - 156 per μ l	14% - 1650 per μ l	Improvement
15..	52	F	U+A.E.	8200	2400	0% - 56 per μ l	4% - 181 per μ l	Improvement
16..	48	M	U	11400	11100	0% - 69 per μ l	38% - 3975 per μ l	No urticaria

*) Abbreviations: U: Urticaria. A.E.: Angioneurotic edema. U.B.: Urticaria bullosa. C.U.: Cold urticaria.

the histamine liberated produces a rise of plasma histamine this may attract eosinophils from bone marrow into blood. Repeated injections of histamine liberators as polymyxin B, regularly give rise to an increasing eosinophilia (Zachariae 1964 a).

The concept of the functions and distribution of the eosinophils may have relevance to therapy. In chronic urticaria for instance a stimulation of eosinopoiesis may lead to an improvement of the urticaria condition. Zachariae (1964 a) injected a potent histamine liberator, polymyxin B, once a week for eight weeks in a group of patients suffering from chronic urticaria. In the majority of patients the urticaria diminished and five of twelve displayed no urticaria during an observation period from two to five months following the cessation of therapy. The clinical improvement was followed by a marked blood eosinophilia. In this study no significant histamine depletion was found to take place (see later chapter XI) and apart from the eosinophilia no other changes were found to occur.

Further investigations on the effect of polymyxin B in patients with chronic urticaria were carried out including another 15 patients. As before,

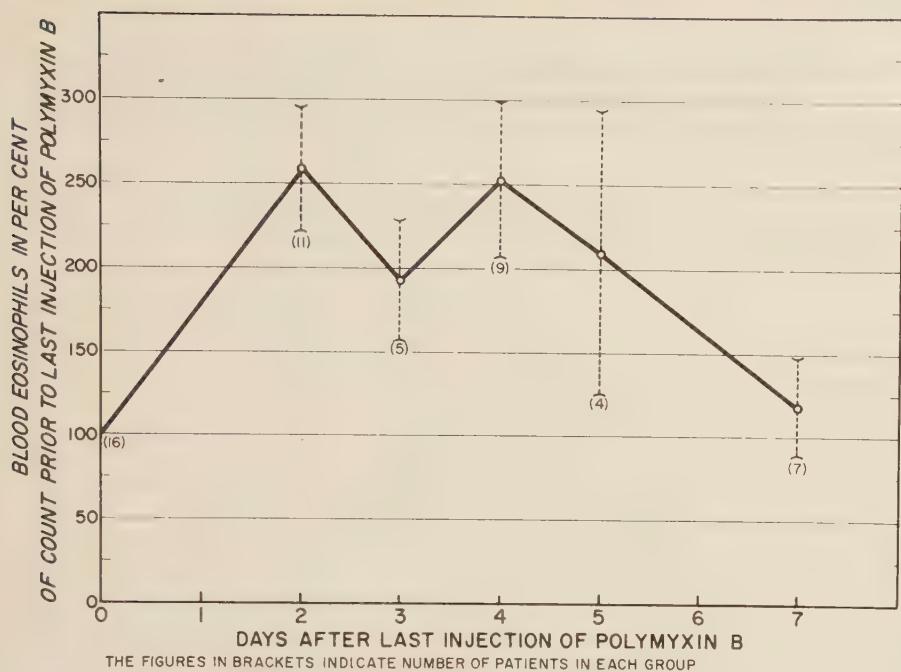


Fig. 13.

Blood eosinophils in per cent of count prior to the last injection of polymyxin B followed after the last of eight weekly injections of 50 mg.

polymyxin B was given intramuscularly in doses of 50 mg once a week over an eight week period, and a marked eosinophilia was observed (table 21). The clinical effect on the disease was not as pronounced as in the earlier investigations (Zachariae 1964 a), still the majority showed improvement after the treatment (table 21). Polymyxin B treatment was also given to a patient, a 21 year old female, with physical urticaria due to cold. In this case the treatment had no effect, although an induced eosinophilia was observed. The absolute count was 106 cells per μl before treatment and 756 per μl after. According to Rorsman (1962) patients with urticaria, produced by physical agents only, show a higher basophil number than other patients with urticaria. The polymyxin B-induced urticaria was in no case accompanied by any basophilia observable in the differential count. No direct basophil counts were performed. In the extended investigations, determinations of 17-keto-steroids and 17-ketogene-steroids were performed in urine prior to and after the treatment showing no apparent influence of polymyxin B.

In 9 cases direct blood eosinophil counts were made prior to the last injection and followed with two or three days interval one week after the last injection (fig. 13) (table 22). The results show (with exception of case 1

Table 22

Blood eosinophil counts before treatment with polymyxin B, 50 mg once weekly for 8 weeks, prior to the last injection, and following the last injection

Case number*)	Eosinophilia (absolute count per μ l)							
	Prior to the last injection	2 days after	3 days after	4 days after	5 days after	6 days after	7 days after	Before treatment
1.....	675		488		506		381	344
2.....	794		844	1350			1169	431
3.....	1300		2781			1806	1813	138
4.....	544	2140		2175				69
5.....	844	994		775			1063	175
6.....	400	1900		1156				163
7.....	425	1544		1094				213
8.....	419		1788	1625				63
9.....	194	569		850		463	488	169
10.....	756	1231	1075					106
11.....	864	1456		1369				269
12.....	131	338			525			94
13.....	325	1025			594			138
14.....	225	469		169			181	56
15.....	3975	3425			1450		700	69

*) Case numbers are the same as in table 21.

from table 22) an increase in the direct eosinophil count with high eosinophil counts from 2 to 5 days after the injection and with a return to almost the same value as prior to the injection. In no case, however, did the value return to or go below the direct blood eosinophil count before the polymyxin B treatment started.

In 8 cases direct blood eosinophil counts were performed one month after cessation of therapy (table 23). The results show that 6 out of 8 patients still had an increase in their blood eosinophil counts in comparison with the counts prior to treatment. The average increase, however, was no longer significant.

Summarizing, the number of eosinophil leukocytes increases in chronic urticaria, treated with polymyxin B, a histamine liberator, given in doses of 50 mg once a week over an eight week period. Special high eosinophil counts were seen from 2 to 5 days after the last injection.

The dose of polymyxin B used does not generally deplete the skin of histamine (see later, chapter XI). It is suggested that the cytoplasmic contents of eosinophils possess antihistaminic activity, which may enable them to neutralize liberated histamine. In certain cases of chronic urticaria, the defence mechanism of eosinophils may be insufficient, but can be activated by repeated injections of polymyxin B.

Table 23

Blood eosinophil counts before and one month after treatment with polymyxin B, 50 mg once weekly for 8 weeks. Case numbers refer to table 21

Case number	Eosinophilia (absolute count per μ l)	
	Before treatment	One month after treatment
2.....	431	688
5.....	175	244
6.....	163	200
7.....	213	125
11.....	106	263
13.....	138	119
15.....	56	100
16.....	69	700
Mean.....	168	305
Standard deviation.....	± 119	± 247
Standard deviation of the mean.....	± 42	± 96

The effects of polymyxin B in chronic urticaria are of great theoretical interest. The additional data gathered from the new cases being reported indicate that polymyxin B is worth trying in this disease, but that it falls short of being a reliable therapeutic agent. Nevertheless, the positive results obtained in the majority of cases make it desirable to continue investigations of the effects of polymyxin B upon eosinophils and histamine in urticaria and other conditions connected with histamine release.

Chapter X

Hormones and Skin Histamine

As early as 1949 *Ragan, Howes, Plotz, Meyer & Blunt* were able to show that glucocorticoids delay the development of the elements of the connective tissue. They found a decreased proliferation of the cells, and a delayed formation of ground substance and fibres. Later *Robertson & Sanborn* (1958) demonstrated a decreased ability of collagen formation in carrageenin provoked granulomas. *Pernokas, Edwards & Dunphy* (1957) and *Trnavsky, Trnavska & Malinsky* (1960) arrived at the same result when studying collagen formation in implanted ivalon sponges and in the wall of turpentine provoked abscesses; while *Jørgensen* (1962) found no significant reduction in collagen content of granulation tissue in open wounds of hydrocortisone treated rats. Also this author, however, demonstrated a reduction of the total amount of produced granulation tissue. Glucocorticoids have also been found to inhibit the development of the inflammatory edema (*Cooper, Schmidt & Schou* 1957), and reduce the water content of the skin of mice (*Hvidberg & Schmidt* 1959). *Asboe-Hansen* (1950 b, 1952), *Cavallero & Braccini* (1951), *Stuart* (1951), and *Fulton & Maynard* (1953) found that mast cells diminished and developed vacuoles during ACTH and cortisone treatment. In addition the number of mast cells decreased. *Bloom* (1952) observed that mast cell tumours in the dog diminished in consequence of cortisone treatment. *Asboe-Hansen* (1958) suggested that a damaging effect of the glucocorticoids on the tissue mast cell in part may explain their therapeutic value in connective tissue disorders.

A relationship between histamine and the adrenal cortex was proposed by *Dale* already in 1920. He found that after adrenalectomy cats become more sensitive to intravenous injections of histamine. Conversely glucocorticoids have been found to protect against the effect of anaphylactic shock (*Telford & West* 1963) and adrenal cortical steroids are now widely used in the treatment of allergic states in which histamine is supposed to act as a mediator.

Goth, Allman, Merrit & Holman (1951) originally suggested that cortisone might delay histamine reaccumulation following depletion. Histamine assays were not performed. Their suggestion was based upon the fact that injections

of Tween 21 into dogs produced a drop in blood pressure, presumed to be due to histamine release; a second injection after one or two days treatment with cortisone produced no similar drop, although this still could be demonstrated in non-treated animals. More recently, cortisone and other glucocorticoids have actually been found to suppress the rate of synthesis of new histamine in rat skin. This was demonstrated *in vitro* (Schayer, Davis & Smiley 1955) and *in vivo* (Schayer, Smiley & Davis 1954). According to Schayer (1963) this does not seem to be due to reduced activity of histidine decarboxylase, but may be a suppression of any one of the steps involved in transport of histidine into the mast cell where decarboxylation and binding occurs. The effects of adrenalectomy in rats are the reverse of those obtained after injection of cortisone (Schayer 1956).

Zachariae (1964 d) studied the influence of betamethasone, a new synthetic steroid, on histamine in human skin. No earlier investigations appear to disclose the changes in histamine content in human skin during therapy with glucocorticoids in therapeutic doses. Daily doses of 2 mg betamethasone markedly reduced the average histamine content of grossly normal skin in 14 patients suffering from various dermatological diseases. It was suggested that lowered histamine content of the skin may partly explain some of the therapeutic effects of glucocorticoids in human skin.

Inhibited connective tissue formation as well as delayed wound healing (Howes, Plotz, Blunt & Ragan 1950, Sandberg & Steinhardt 1963, Saviof & Dunphy 1954, L. Zachariae 1954), have been reported as severe side effects during glucocorticoid treatment. As histamine release may be a part of tissue response to injury and thereby a stimulus of repair, and because reparative growth as maybe all rapid tissue growth may be requiring histamine (see chapter V) these side effects also in part may be explained by the action of glucocorticoids upon histamine.

The idea that glucocorticoids inhibit the biogenesis of histamine appears to be the most acceptable explanation of the relation of glucocorticoids to the inhibition of histamine activity. However, this explanation does not militate against the opinion that glucocorticoids inhibit extravasation and stasis of tissue fluid by direct vasoconstriction (Menkin 1953, Stefanini & Dameshek 1955) nor against the theory that they suppress some mechanism which has deterrent effect on the activity of histaminase (Kelsall & Crabb 1959). In favor of the latter suggestion are reports that adrenalectomy or hypophysectomy destroy 70 to 80 per cent of the extractable diamine oxidase (histaminase) in cats (Hæger, Jacobsohn & Kahlson 1952).

The question as to whether glucocorticoids besides restricting the rate of histamine formation, may also act as histamine liberators, has not yet been thoroughly investigated. In rat skin, however, the studies of Telford & West (1960 b) indicate the latter suggestion not to be valid. They found no loss

of histamine from the rat skin after four days of treatment with various glucocorticoids, although, when the treatment was extended to nine days, the skin was depleted of its histamine. For example, only 12 per cent of histamine remained in the skin after treatment with fludrocortisone. With prednisolone the histamine content of abdominal rat skin was reduced to 55 per cent of the control value.

Adrenalin possibly antagonizes histamine production by inhibiting histidine decarboxylase from converting histidine to histamine (*Wenzel & Rosenberg* 1956). Ascorbic acid, through potentiating adrenalin has been suggested to possess a similar effect upon histaminogenesis (*Wenzel & Rosenberg* 1956). The effect of these substances upon skin histamine, however, has not been investigated.

The effect of the pituitary thyrotropic hormone on connective tissue is in many ways the opposite of that of cortisone (*Asboe-Hansen & Wegelius* 1960). *Asboe-Hansen & Iversen* (1951) showed that the number of mast cells increased in the guinea-pig's retrobulbar connective tissue after thyrotropin administration. One year earlier, *Asboe-Hansen* (1950 a) had observed an increase in the number of mast cells in the presence of myxedema. *Wegelius & Asboe-Hansen* (1956) studied the effect of hormones on living mast cells of the hamster's cheek pouch. Cortisone caused degranulation and vacuolisation of the mast cells, while thyroxine caused a reduction in the size of the mast cells and their degranulation in free connective tissue. *Leger & Masson*, in 1948, noted that injections of thyroid extracts produce a striking modification of the anaphylactic reaction in rats given intraperitoneal injections of egg-white. The reaction in untreated rats was characterized by edema of the extremities with recovery within six hours, while a shock-like condition was produced within a few minutes after the egg-white injection in the thyroxine treated rats. *Parrat & West*, in 1960, confirmed this result. *Spencer & West* (1961) found that injections of thyroxine sodium increased the sensitivity of mice to injected histamine, the maximal effect occurring after six days. Hydrocortisone antagonised this effect of thyroxine. If the treatment was continued beyond six days, the adrenal glands enlarged and its secretion probably accounted for a moderate decline in the histamine sensitivity. *Gotzl & Dragstedt* (1954) and *Feldberg & Loeser* (1954) found the histamine content of the skin to be high in hyperthyroid states in rat and man, whereas the skin histamine was low in myxedema. The present author only examined skin histamine in one case of myxedema, a five year old girl. In this case the skin histamine was low, i. e. 5.1 µg/g, the determination performed on dried defatted skin from the forearm.

It is well known that young children afflicted with atopic dermatitis have a tendency to outgrow this disease during the changes of puberty. It is equally well known that chronic urticaria in women has a tendency to appear

about the time of the menopause. This may indicate an effect of sex hormones on histamine. As most of the effects of hormones on mast cells and histamine are indirectly mediated (see *Kelsall & Crabb* 1959), it is, however, difficult to determine the part actually played by the hormones.

Estrogen has a number of complex and diverse physiological actions in addition to producing estrus or the menstrual cycle. Apparently, with the exception of certain metabolic relations, the chief capacity enabling estrogens to perform their multiple and varied actions is dependent upon its ability to produce and maintain hyperemia in certain "target structures" and restrict permeability of the connective tissue ground substance until the purpose has been achieved (*Duran-Reynals & McCrea* 1953).

With estrogen treatment of mice *Arvy* (1955) found an increase in the number of the mast cells in the subcutaneous tissue. *Schiff & Burns* (1961) studying biopsy specimens from the mucous membrane of patients undergoing tonsillectomy, noted an increased number of tissue mast cells in patients receiving conjugated estrogen intravenously before the operation. The authors concluded that conjugated estrogens appear to exert a protective effect on tissue mast cells with increase in cell count and granule storage. Conversely, *Winer, Bierman & Sternberg* (1963) found that topically applied estrogen stimulated degranulation and production of acid mucopolysaccharides by mast cells in the skin of hairless mice. None of these authors determined histamine and in the latter study no control studies were performed with regard to an influence of the vehicle carrying the hormone. Histamine determinations, however, were done by *Telford & West* (1960 b) in rats treated with estrogen.

Telford & West (1960 b) also investigated the effects of testosterone treatment on rat skin. As with estrogens they found a weak adrenal corticoid-like activity lowering skin 5-hydroxytryptamine, but not skin histamine. As mentioned earlier, in rats, however, the functions of histamine may in part have been pre-empted by 5-hydroxytryptamine (*Benditt, Holcenberg & Lagunoff* 1963), so whether or not androgenic hormones are capable of altering skin histamine levels has not been thoroughly investigated.

In the studies by the present author no special investigations were performed in order to investigate the influence of sex hormones on skin histamine. It may, however, be noted that in the studies on histamine in grossly normal human skin, no difference was found between the skin histamine values of males and females (*Zachariae* 1964 b).

It is concluded that glucocorticoids reduce the histamine content of skin in rat and man. In rat, histamine formation is reduced. The mode of action on skin histamine in man has not yet been thoroughly investigated. The influence of adrenalin, the thyrotropic hormone, thyroxine, and sex hormones upon skin histamine still remains to be elucidated.

Histamine Liberators and their Influence on Skin Histamine

Histamine liberator is the name commonly applied to chemical substances supposed to possess a strong histamine releasing capacity, often beyond their other pharmacological effects. *Paton* (1956) reserved the term to one of seven groups of various substances capable of releasing histamine from the tissues and to which he assigned compound 48/80 and the dibasic and polybasic histamine-releasing compounds. He characterized the histamine liberators as compounds having no direct action on the tissues, initiating a rapid process of histamine release, and acting with particular effect on skin and muscle. The name, however, usually is applied more freely. Among the best known histamine liberators are compound 48/80 (*Paton* 1951), which is a polymeric condensation product of paramethoxyphenethylmethylamine and formaldehyde, polymyxin B (*Bushby & Green* 1955), a basic polypeptide, and compound L 1335 (*Feldberg & Lecomte* 1955), an equimolecular mixture of hydroxyphenylhydroxymethylphenylmethylpropylamine and its dehydroforms. A better name than histamine liberators seems to be mast cell depleters, as at the same time as they release histamine in the body, they may release other chemical substances for example hyaluronic acid (*Asboe-Hansen & Wegelius* 1956). In the dog early reports suggested that they liberate heparin (*McIntosh & Paton* 1949). In the rat and the mouse histamine liberators release 5-hydroxytryptamine (*Parrat & West* 1957 b, *Telford & West* 1960 a), while in the cat, dog and rat, if a fairly large dose of histamine liberator is given, there appears in the plasma or incubation fluid what one refers to as "slow reacting substances" (*Paton* 1956, *Uvnäs & Thon* 1959), corresponding to similar substances appearing in anaphylaxis (*Beraldo* 1950). The name mast cell depleters has been suggested by *Bhattacharya & Lewis* (1956), but is rarely used, partly because our knowledge of the subject is growing so rapidly that additional name giving seems premature; partly because the name may convey the misunderstanding that these substances rid the mast cells not only of all their histamine, but also of any other active substance within them (see *Paton* 1957).

Besides releasing histamine, histamine liberators may also increase histidine decarboxylase activity and histamine synthesis in skin (*Schayer, Rothschild & Bizóny* 1959).

Various tissues show different degrees of resistance to different histamine liberators, not only in the same animal but also in other species. For example compound 48/80 releases large amounts of histamine in the rat (*Bushby & Green* 1955), but appears not to be a strong histamine liberator in the mouse and the hamster (*Riley & West* 1955 b, *Parrat & West* 1957 a). The same appears to be true of polymyxin B (*Parrat & West* 1957 a). While compound L 1935 possesses a rather weak activity in the rat, it is highly active in the mouse and the hamster (*West* 1958). The guinea-pig is strongly resistant to compound 48/80 (*Mota & Wugman* 1956, *Boreus* 1960). The danger of drawing too far-reaching conclusions from animal experiments should be pointed out. However, all these substances mentioned above seem to be able to release histamine in man (*Lecomte* 1957, *Nordlander* 1957, *Meneghini & Levi* 1959, *Lecomte* 1959, *Zachariae* 1964 e).

Zachariae studied local (1964 e) and systemic (1964 a) effects of polymyxin B. Polymyxin is the name applied to a group of antibiotics derived from various species of bacillus polymyxa. Five polymyxins designated by the suffixes A to E are known. Chemically the polymyxins are basic polypeptides. Polymyxin B has a molecular weight of 1150 ± 50 and an empirical formula of $C_{56}H_{99}N_{16}O_{14}$ (*Jawetz* 1956). It contains the aminoacids leucine, phenylalanine, threonine, and, α , λ -diaminobutyric acid in addition to a fatty acid, d-6-methyloctan-1-oic acid. Commercial preparations are 65 to 75 per cent pure. They appear as yellowish scales that are readily soluble in water or saline up to concentrations of 25 mg per ml. Polymyxin A, C and D are no longer in clinical use, as they are liable to cause temporary damage to the renal tubules, while polymyxin B and E given in normal doses are considered not to possess any serious toxicity (*Swift* 1953, *Swift & Bushby* 1956, *Jawetz* 1956, *Jawetz* 1961). Only at dose levels greater than 3 to 4 mg per kg per day, there may be a reduction in creatinine clearance and glomerulus filtration rate (*Jawetz* 1961). These disturbances appear reversible if treatment with excessive doses is not prolonged into the development of frank renal failure. At parenteral dose levels of 2.5 mg per kg per day there may also be observed proteinuria and cylindruria occasionally accompanied by an increase in white, red, and epithelial cells in the urinary sediments (*Jawetz* 1961).

In the studies of the author no patient received higher doses than 50 mg per day, commonly only 50 mg once a week, and with the exception of a transient proteinuria, observed once in one patient, no signs of nephrotoxic effects were observed.

In human skin intracutaneous injection of polymyxin B produces a weal

Table 24

Reactions to a second intracutaneous injection of 50 mg polymyxin B following a previous injection producing a large weal

Case no.	Sex	Age	Diagnosis	Interval between injections	Second reaction
1.....	F	60	Neuroderdermatitis	24 hours	Slight erythema
2.....	F	72	Drug eruption	24 hours	Slight erythema
3.....	F	55	Intertrigo	24 hours	Slight erythema
4.....	M	62	Parapsoriasis	3 days	Erythema and small wealing response
5.....	M	43	Psoriasis	3 days	Slight erythema
6.....	M	44	Psoriasis	3 days	Erythema
7.....	M	67	Varicose ulcer	4 days	Local urticaria
8.....	M	22	Acne vulgaris	4 days	Local urticaria
9.....	M	22	Impetigo	4 days	Local urticaria

of the same type as that following an injection of histamine (*Fekete & Kalz* 1962, *Zachariae* 1964 e). This is similar to injections of 48/80 (*Bernstein & Feinberg* 1956, *Meneghini & Levi* 1959, *Fekete & Kalz* 1962) or L 1935 (*Lecomte* 1959). Repeated injections of 50 mg polymyxin B into the same skin area, produce no weal 24 hours after the first injection, either a weakened reaction or no urticarial response 3 days after the first injection, but an almost normal or entirely normal reaction 4 days after the first response (table 24). This is in agreement with analyses of the histamine content in grossly normal human skin prior to and after injections of 50 mg polymyxin B into the skin (*Zachariae* 1964 e). Intradermal injections of polymyxin B markedly reduced the histamine content in grossly normal skin of patients suffering from various skin diseases, when examined one hour after the injections. No significant alterations in skin histamine was found in other patients receiving physiologic saline, nor in patients studied four days after intradermal injections of polymyxin B (fig. 14).

When histamine release is produced experimentally by means of histamine liberators, a simultaneous degranulation of mast cells occurs in most cases (*Riley* 1963, *Fawcett* 1954, *Riley & West* 1955 a, *Veil* 1956, *Hunt & Hunt* 1956, *Boreus* 1960). The degranulation of mast cells following injection of compound 48/80 was observed by *Wegelius & Asboe-Hansen* (1957) in living cells of the hamster cheek-pouch. These workers, however, also found that injections of histamine or of physiological saline produced a similar degranulation. Consequently they suggested that in all cases the degranulation was provoked by an increase of tissue fluid. *West* (1956) observed that smaller doses of histamine liberators could reduce the histamine content in the tissues of rats to approximately 30 per cent of normal values without

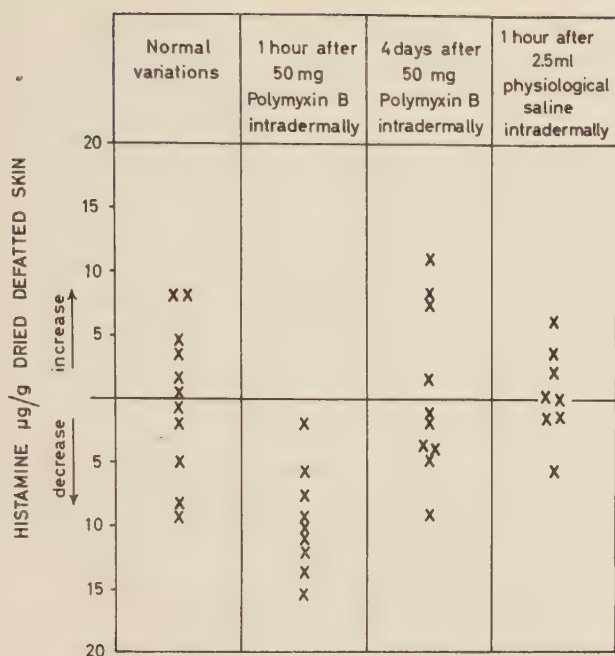


Fig. 14.

Local alterations in skin histamine in patients with various skin disease treated with intradermal injections of 50 mg polymyxin B dissolved in 2.5 ml physiologic saline or with intradermal injections of 2.5 ml physiologic saline compared with normal variations in skin histamine from the same area.

any morphological changes of the mast cells being observed. Also *Smith* (1958), when studying histamine release from peritoneal mast cells in rats, could confirm that secretion by the mast cell does not necessarily require degranulation or other visible cytological change of the cells, this indicating a merocrine nature of mast cells.

The clinical syndrome of anaphylaxis differs considerably from one species to another. Nevertheless the action of histamine liberators in most species examined so closely resemble anaphylactic shock of the same animal, that this indicates that the pharmacologically active substances responsible for the syndrome are released from mast cells (*Mongar & Schild 1952, Riley 1953, Riley & West 1955 b, Keller 1957, Akcasu & West 1960, Amann 1961*) (see also chapter VI). Also *Högberg & Uvnäs (1960)* have shown that the effects of compound 48/80 and of the antigen-antibody reactions on tissue mast cells of rat mesentery are similar.

Raab (1962) studied the effect of compound 48/80 on the experimental dinitrochlorobenzene contact dermatitis of the guinea-pig. In approximately 50 per cent of the animals, it was possible to prevent an eczematous reaction,

when the animals received 1 mg of compound 48/80 intraperitoneally daily for 10 days following sensitization until the reapplication of the sensitizing agent. The other half of the animals showed a diminished eczematous reaction in comparison with the control animals. When 0.5 mg compound 48/80 was given locally as a subcutaneous injection prior to the reapplication of the sensitizing agent the eczematous reaction was prevented in all cases. Inspired by this report the present author tried to prevent a positive reaction in a patch test with rubber on a patient, a 61 year old female known to be allergic to rubber. 50 mg Polymyxin B was given locally as an intradermal injection prior to the application of the test-substance, 24 hours later, another 50 mg was given as a subcutaneous injection immediately under the patch test. When the test substance was removed 48 hours after the application, the skin, however, showed a typically positive eczematous reaction with erythema, infiltration and vesicular response, not to be mistaken for the urticarial weal previously produced by polymyxin B. *Meneghini & Levi* (1959) had previously found that epicutaneous reactions were not influenced by local injections of 48/80, while cutaneous reactivity of the tuberculin-type was partly inhibited by the histamine liberator.

As mentioned before, healing after wounding measured either quantitatively by determining the tensile strength in situ or by formation of collagen, was found increased in rats by compound 48/80 or polymyxin B by some workers (*Kahlson, Nilsson, Rosengren, and Zederfeldt* 1960, *Hvidberg, Jorgensen, Schmidt & Schou* 1961, *Sandberg* 1963), but not found influenced by polymyxin B by others (*Fiore-Donati & Moltke* 1960), and decreased after 48/80 or polymyxin B by still others (*Boyd & Smith* 1959, *Fenton & West* 1963). The experimental conditions in the different investigations, however, were far from identical. *Fiore-Donati & Moltke* (1960) and *Fenton & West* (1963) also studied the effect of reserpine, a 5-hydroxytryptamine liberator, upon the tensile strength of the healing incision wounds in rats, and both investigations revealed that reserpine caused a significant decrease in the tensile strength of the wounds. In this connection, again the attention must be drawn to the fact that rats and mice seem special among the species in having demonstrable quantities of 5-hydroxytryptamine along with histamine in their mast cells, and that rats and mice are very resistant, when compared to the guinea-pig, rabbit, and man, to the effects of histamine, while rats have been found quite susceptible to the vascular permeability increasing effects of 5-hydroxytryptamine (*Sparrow & Wilhelm* 1957). These facts must also be considered, when evaluating the possible influence of polymyxin B on experimental skin tumours in mice. This was studied by the author in a limited number of mice, 34 animals totally, with experimental skin tumours induced by painting the skin with 9,10-dimethyl-1,2-benzanthracene (see also chapter V).

A total of 40 albino mice of strain Strit Leo, weighing approximately 22 g, were painted once weekly for 6 weeks in the interscapular region with 0.05 ml of a 0.25 per cent solution of 9,10-dimethyl-1,2-benzanthracene. At the end of 6 weeks 34 mice showing skin papillomas were selected. None of the growths showed macroscopic signs of malignancy at the time of selection. Twenty of the mice were treated with polymyxin B, injected intraperitoneally twice a week. The dose was 0.2 ml of a solution containing 0.2 mg per ml. The remaining fourteen mice received injections of 0.2 ml physiological saline intraperitoneally. Both groups were treated for 5 weeks. One week after the last injection the mice were killed. The tumours were removed and weighed. As little material as possible was selected for microscopic examination, while the rest was determined for histamine.

In the polymyxin B treated group, consisting of twenty mice, two mice developed skin carcinomas, in three other the growths were suspected of beginning malignancy, two only revealed epithelial hyperplasias, while the remaining mice all seemed to have skin papillomas. In two of these mice the material selected for microscopic examination was too scarce to allow any diagnosis. They were, however, as the other papillomas, relatively soft pedunculated without peribasal infiltration, while the carcinomas were firm, nodular and on a broad base surrounded by cutaneous and subcutaneous infiltration. In the control group, consisting of fourteen mice, two mice developed skin carcinomas, one was suspected of beginning malignancy, six had non-malignant skin papillomas, while the remaining five only revealed epithelial hyperplasias.

As summarized in table 13, table 14, and table 15 (p. 45 and 46) the data show that the group of mice treated with polymyxin B does not differ significantly from the control group concerning weight, histamine content, and estimated quantity of mast cells of their tumours. Table 25 shows that also the proportion of tumours showing malignancy or suspected malignancy is almost identical for the two groups. It is suggested that the possible effect of histamine liberators on skin tumours should be studied using compound L 1935, a more potent histamine liberator in the mouse (*West* 1958), or still better in other species than the mouse or rat, where the functions of histamine possibly in a large part have been preempted by 5-hydroxytryptamine.

In inflamed skin, compound 48/80 produces a weaker response than in normal skin (*Reed, Kierland & Code* 1958). In skin damaged by X-rays *Eisen, Ellis & Wilson* (1956) found no reaction at all. On the other hand *Maragnani* (1960) could demonstrate an increased reaction in mastocytosis. In patients with acute urticaria even very small amounts of compound 48/80 may elicit a triple response (*Juhlin & Rune* 1962).

Table 25

Proportion of various tumour groups in mice with experimental skin tumours induced by painting the dorsal skin once weekly for six weeks with 9,10-dimethyl-1,2-benzanthracene and thereafter treated with either 0.05 mg polymyxin B in 0.2 ml physiological saline or with 0.2 ml physiological saline alone intraperitoneally twice a week for 6 weeks

Tumour group	Polymyxin B treated		Controls	
	Number	Per cent	Number	Per cent
Carcinomas.....	2	10.0	2	14.3
Skin papillomas suspected for beginning malignancy.....	3	15.0	1	7.1
Malignant + suspected.....	5	25.0	3	21.4
Non-malignant skin papillomas.....	13	65.0	6	42.9
Epithelial hyperplasias.....	2	10.0	5	35.7
Non malignant totally.....	15	75.0	11	78.6

Urticaria has been induced locally by quantitative iontophoresis of 48/80 (Juhlin 1962). The threshold dose of 48/80 causing urticaria was found to be decreased in acute urticaria and in atopic dermatitis.

In a further effort to investigate whether patients with urticaria or atopic dermatitis differ in sensitivity to histamine liberators, Juhlin (1963) administered compound 48/80 by continuous infusion through two hours to patients with atopic dermatitis, chronic urticaria, and alopecia areata. The time for the appearance of urticaria varied widely. Patients with chronic urticaria apparently tolerated the infusion best. In cases of alopecia areata the urticaria first appeared in the bald areas of the scalp, which were rich in mast cells. In all patients, the white blood cells increased after the infusion, with appearance of juvenile leukocytes. The eosinophils showed extrusion of the granules, and in nine out of the fourteen patients the number of basophils increased during infusion, owing to the appearance of young, non-degranulated cells. Except for severe itching experienced by the patients with atopic dermatitis, the 48/80 infusion was tolerated with little discomfort. A slight fall in systolic blood pressure occurred in most of the subjects, and some patients experienced drowsiness for some hours.

For the last several years, histamine liberators have been tried in clinical studies on patients suffering from a number of different diseases. Doses from 1 to 10 mg of compound 48/80 or from 25 to 100 mg polymyxin B have been used by different workers. Nordlander (1957) using 48/80 reported immediate reactions with erythema, edema, and urticaria as well as a later and beneficial effect upon the syndrome "restless legs" and also upon a case of atopic dermatitis. Under cover of antihistaminics (Sicuteri, Ricci,

Monfardini & Ficini 1956, *Sicuteri & Monfardini* 1958, *Halpern* 1958, *Sicuteri, Michelacci & Franchi* 1963) treatment with compound 48/80 has been reported effective in urticaria, allergic dermatitis, vasomotoric rhinitis, and bronchial asthma. Intra-carotid administration of 48/80 has been used against resistant migraine and epilepsy (*Sicuteri, Michelacci & Franchi* 1963). The same authors also used the drug with success in patients with angina pectoris. *Asboe-Hansen* (1961) found that, in a patient with urticaria pigmentosa and generalized mastocytosis, polymyxin B, when given continuously in doses of 100 mg daily, brought about a partial or complete degranulation of mast cells, a subsidence of skin urtication, a decrease in liver size and ascites, and a considerable increase in the count of basophils.

In none of the above-mentioned studies, skin histamine was determined. This, however, was studied by *Zachariae* (1964 a) in grossly normal skin of patients with chronic urticaria treated with polymyxin B, given intramuscularly in doses of 50 mg once a week over an eight week period. In one patient, a child, after two injections, the dose was reduced to 25 mg weekly since rather severe angioneurotic edema followed the first two injections. No significant changes were found in the histamine content of grossly normal skin during or after the treatment with polymyxin B in these doses (table 26). In agreement with polymyxin B's quality as a histamine liberator some side-effects were demonstrated. Local irritation and pain at the site of the injection (the gluteal area) was noted, the pain often radiating peripherally. A generalized urticaria usually occurred from two to six hours after the injections. Other side-effects were nausea, dizziness and a feeling of weakness and drowsiness. Occasionally paresthesia and hypesthesia also developed and almost all patients complained of tingling of the tongue as well as of a taste of metal. Four patients out of twelve developed fever in connection with the injections. Gastrointestinal disturbances as vomiting and diarrhea were rare, but were noted in two cases. None of the patients of this study showed nephrotoxic effects. Proteinuria, haematuria, or cylindruria were not observed, and serum kreatinine was persistently normal in all patients. The electrocardiograms displayed ventricular extrasystoles in two patients on the day of administration of polymyxin B. The systemic side-effects were considerably more pronounced in patients suffering from chronic urticaria, than in patients suffering from varicose leg ulcers, psoriasis, cutis hyperelastica, or condylomata, receiving the same dose of polymyxin B. As mentioned in chapter IX, although the patients displayed no significant skin histamine depletion (table 26), the urticarial condition of the majority of the patients treated with polymyxin B, was improved. The clinical improvement was accompanied by a marked eosinophilia. It was suggested that the eosinophilia reflected a direct effect of histamine upon eosinopoiesis, and that, in certain cases of chronic urti-

Table 26

Histamine levels in grossly normal skin of patients with chronic urticaria before treatment with polymyxin B, 50 mg once weekly for 8 weeks, during the first week of treatment, prior to the last injection, and following the last injection

Case no.	Age	Sex	Skin histamine (μ g per gram dried and defatted skin)										
			Prior to the first injection	3 hours after	6 hours after	1 day after	2 days after	7 days after	Prior to the last injection	3 hours after	1 day after	2 days after	7 days after
1...	69	M	22.6	20.3	+	22.0	17.1	20.4	9.7	9.7	7.3	9.8	19.4
2...	31	F	40.7	40.2	+	32.5	28.8	30.0	18.6	21.2	28.7	35.6	+
3...	48	F	19.5	+	17.3	17.8	22.0	16.9	31.1	12.7	32.4	35.9	33.8
4...	67	F	25.2	+	23.9	33.8	32.8	25.7	27.5	20.8	29.4	31.0	28.1
5...	47	M	28.9	+	36.2	19.4	33.4	25.7	42.4	33.9	42.0	40.3	33.1
6...	31	F	11.0	6.3	+	16.0	13.9	21.0	16.7	14.6	22.0	20.3	+
7...	18	M	19.9	12.2	+	42.3	36.4	20.2	17.6	21.0	16.1	13.8	23.7
8...	12	M	33.7	+	26.9	31.1	28.6	32.8	36.3	42.0	32.2	38.3	34.3
9...	29	M	26.3	27.9	+	24.2	29.0	26.0	31.9	29.6	24.4	33.9	14.9
10...	48	M	36.1	+	+	+	+	+	47.2	+	+	+	+
11...	12	M	36.1	+	+	+	+	+	68.3	+	+	+	+
12...	17	M	31.2	+	+	+	+	+	28.2	+	+	+	+

+) Not determined.

caria, the defence mechanism of the eosinophils may be insufficient. It was also suggested that this defence mechanism of eosinophils may be stimulated by repeated injections of polymyxin B given in moderate doses.

When reexamining the patients with chronic urticaria, treated with polymyxin B over an eight week period, it was observed that commonly the scars left after punch biopsies performed during the treatment appeared larger than what is normally found using the same technic. In several cases the scars were keloidal (fig. 15). This agrees with the increased formation of collagen (hydroxyproline) found by *Sandberg* (1963) in rats after preoperative treatment with the histamine liberators 48 80 or polymyxin B.

It was also observed that some scars were surrounded by a several millimeter broad dark brown pigmented area (fig. 15). The investigations do not warrant any conclusions concerning the mechanism by which this melanin pigmentation is formed. However, it is tempting to stress the similarity with the pigmentation in human mastocytosis of the skin, where we also find an increase in histamine formation and liberation. So far, no explanation has been given of the melanin pigmentation of urticaria pigmentosa.

Summarizing, histamine liberators release histamine from mast cells. At the same time they may also release other more or less active substances within these cells i. e. hyaluronic acid, heparin, 5-hydroxytryptamine, and "slow reacting substances". Great species variations exist. Nevertheless the

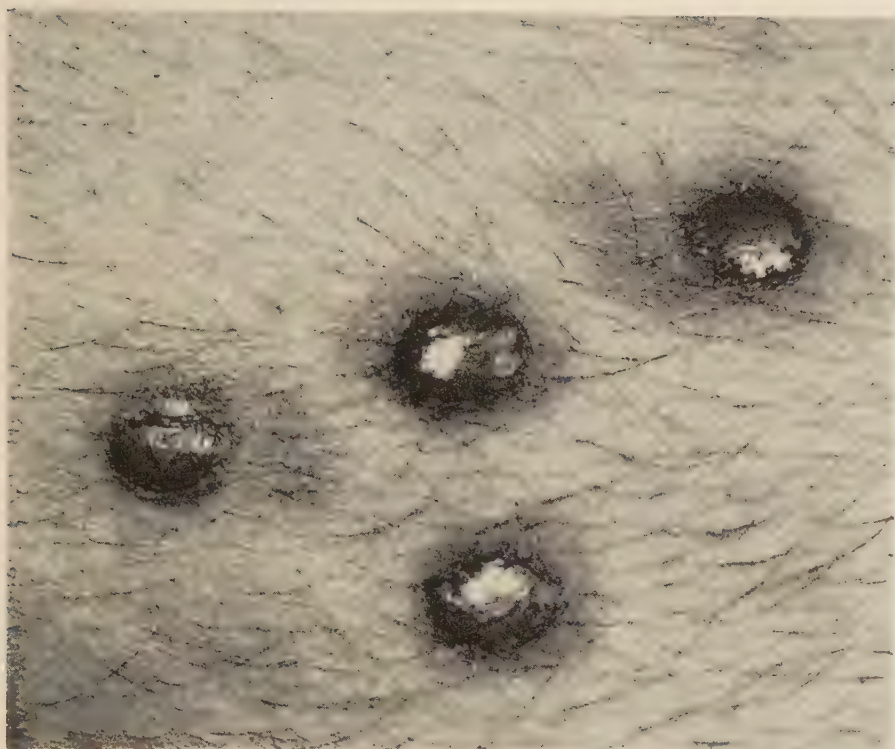


Fig. 15.

Keloids in a patient with chronic urticaria after punch biopsies performed during treatment with 50 mg polymyxin B, once weekly for 8 weeks. Note the dark brown pigmented area surrounding the scars.

action of large doses of histamine liberators in most species closely resemble anaphylaxis in the same animal. Histamine liberators have been in use during the last decade in experimental studies on tissue injury, repair, inflammation, tumour growth, and immediate and delayed types of allergy. Besides they have been tried in clinical studies on patients with various diseases.

The author studied local and systemic effects of the histamine liberator polymyxin B in man and the possible influence upon experimental skin tumours in mice. In the latter investigations no significant effect of polymyxin B was demonstrated. The systemic effects in man were all in agreement with polymyxin B's quality as a histamine releasing agent. Intradermal injections of 50 mg polymyxin B produced an urticaria weal and a considerable reduction in skin histamine. Repeated injections of the same dose produced a weaker response the first days after the injection. Four days after, however, it was possible to reproduce a local urticarial reaction. This

was in agreement with analyses of skin histamine which showed normal values four days after intradermal injections of 50 mg polymyxin B. Repeated injections of 50 mg polymyxin B, given once a week intramuscularly to patients suffering from chronic urticaria, improved the clinical condition in the majority of patients, even if no significant skin histamine reduction was demonstrable. The clinical improvement was accompanied by an eosinophilia.

Chapter XII

General Conclusions

It has been realized for many years that histamine occurs normally in skin. Histamine is also known to participate in anaphylactic skin reactions of experimental animals. In spite of this, knowledge has been scarce about the functions of skin histamine in man and the factors which influence it. A review of the literature has revealed little concerning skin histamine under pathological conditions, and so far, it has not been established whether histamine is of physiological importance in skin. The present investigations were undertaken in an attempt to elucidate the significance of changes in skin histamine and to afford a survey of the role of histamine in skin.

It was necessary to investigate normal skin. Species variation were already known and regional differences in skin histamine had been reported in experimental animals. The investigations of the present author revealed skin histamine in man to follow the same pattern as in experimental animals. It is concluded that, when studying skin histamine, besides species variations also regional variations, the age of the individual, and individual variations should be considered. The author's investigations reveal, that in man, skin histamine is low in early fetal life, but rises with increasing fetal age. In the adult, skin histamine slowly decreases, although this decrease is only moderate.

In the studies on human fetal skin, the investigations demonstrated a high ratio of wet weight to dry defatted weight which declined with increasing fetal age. *Kahlson* (1962) associated the general processes of growth and repair with high histamine forming capacity. From the results of the present author it seems likely that in early fetal life characterized by rapid tissue growth the demand for histamine may be so great that a storage of cell-bound histamin can scarcely take place simultaneously. The same seems to be the case in reparative growth and possibly also in some cases of malignant growth. This was, however, only studied by the author in experimental animals and not in man, and the investigations concerning

malignant growth, should be considered preliminary. The work, mentioned here, does not disagree with the theories of *Kahlson* (1962) and are in accordance with the concept of *Asboe-Hansen, Dyrbye, Moltke, and Wegelius* (1959) that tissue edema initiates any process of growth, regeneration, and repair of connective tissue (see also *Asboe-Hansen* 1963 a). The observations that mast cells were almost not to be found in early human fetal skin and in fresh granulation skin tissue of pig, that mast cells diminished in skin grafts after operation, and when experimental skin papillomas in mice turned malignant, all agree with the suggestion that in growth and repair of connective tissue the demand for mast cell constituents, among these histamine, is great. It is believed that this is the trail to be followed when seeking the possible physiological importance of histamine in skin as well as in connective tissue in general. To-day evidence is not complete. Many of these findings may only be secondary reflections of changes occurring in the connective tissue.

The observations on skin histamine in urticaria, urticaria pigmentosa, experimental allergic contact dermatitis, and erythema multiforme suggest that histamine may play a part in these skin diseases. Already the fundamental studies by *Lewis & Grant* (1924) made it likely that histamine was of pathogenetic importance in urticaria. This is still valid. When depleting a skin area of histamine a new urticarial reaction can not be reproduced for some time. Whether histamine is of pathogenetic importance to the other skin diseases mentioned, is not known, with the only exception of the wealing response in urticaria pigmentosa. As in repair, the findings may be only secondary reflections of changes occurring in the connective tissue.

To-day, however, we possess tools for further investigations; it is possible with both corticosteroids and histamine liberators to influence skin histamine. The present results show that the relative effects of corticosteroids in decreasing the histamine level in human skin parallels their effects in therapy. Histamine liberators have been in use during the last decade in experimental work and have been tried in clinical studies on patients with various diseases. Much research, however, is still left to be done, till these substances can be of general use in medicine.

It is a common misunderstanding to identify urticaria with allergy. The same regards eosinophilia. Urticaria is nothing but the visual effect of histamine liberation in skin. In any individual it is possible to produce an urticarial reaction by introducing a histamine liberator into the skin. Eosinophils seem to accumulate where histamine is liberated. It must be remembered that histamine participates in anaphylactic reactions. We therefore often record eosinophilia and allergic reactions in the same individual. Whether or not eosinophils are wandering cells capable of inactivating histamine locally on demand has not been proved. Nevertheless the results obtained,

i. a. in the present study, make it desirable to continue work along these lines.

As mentioned, histamine is not the only inhabitant of the tissue mast cell, and not the only substance liberated by histamine liberators. The same applies to liberation by injury and in various clinical conditions in which histamine may have a part to play. Efforts should be taken to investigate the presence and behaviour of other mast cell substances in skin.

Histamine release and histamine increase have been valuated by the variations in the content of histamine in skin. It is suggested that the work should be continued in connection with investigations concerning histamine turnover in various tissues.

Chapter XIII

Summary

The increasingly intensified research on skin and the recognition of the important part played by histamine as an edema producing substance has necessitated further studies on histamine in normal and diseased skin. As mentioned in the *introduction*, the spectrofluorometric assay method has made possible the measurement of histamine in small skin biopsies.

The object of the present study was to investigate the levels of skin histamine in normal and diseased skin, to attempt to elucidate the significance of changes in skin histamine, and to afford a survey of the role of histamine in skin.

In *chapter I* a brief review is given of the chemistry and metabolism of histamine, β -imidazolethylamine. Biologically, histamine is formed by enzymatic decarboxylation of histidine. Histamine exists in the body in three forms: Free histamine, bound histamine, and conjugated histamine (acetylhistamine). The two first mentioned are the important. It is in its free form that histamine is able to produce its effects, but normally, very little free histamine is present in the fluids and intercellular spaces of the body, including skin. Almost all histamine determined consists of bound histamine. Most bound histamine is found within the mast cell. The mechanism of binding is not well understood. After its formation and binding, intracellular histamine is released slowly from the tissues. Histamine release may also take place rapidly after stimuli of chemical or physical nature. Histamine is either excreted unchanged in the urine or excreted after degradation to methylhistamine, imidazole acetic acid, methylimidazole acetic acid or acetylhistamine. Methylation of histamine followed by oxidation of the formed methylhistamine, and oxidation of histamine seem to be the main metabolic pathways.

Chapter II describes the methods used by the author. Particularly the spectrofluorometric assay method. The initial experiments to evaluate this method are recounted. Almost all determinations were made on defatted and dried skin. Defatting was carried out, to avoid possible errors related

to sebaceous glands or residual amounts of subcutaneous fat. It was considered important to calculate values on a dry weight basis when determining an edema-producing substance like histamine. Most skin samples were removed under local anaesthesia. The influence of the local anaesthesia was investigated and found to be negligible. The recovery of histamine was investigated and found to range from 86 to 110 per cent, with an average of 96.2 per cent. The weak step in all methods for determining histamine in tissues is the separation of histamine from histidine. Therefore the recovery of histidine was investigated. It was found to be 0 per cent. To support the identification of histamine, excitation spectra, fluorescence spectra, and some solubility characteristics were investigated and found to correspond when using authentic histamine and apparent histamine from skin. Skin histamine levels determined by spectrofluorometry in various species were in accordance with results by other investigators obtained by bio-assay or spectrophotometry. It is concluded that the spectrofluorometric method offers a simple, accurate, and sufficiently specific assay of skin histamine.

Where histologic studies were performed on skin, the biopsies were fixed in an aqueous lead subacetate solution and stained with toluidine blue. The leucocytic response to aseptic inflammation in urticaria pigmentosa was studied using a "skin window" technic. The "skin window preparations" were stained using the May-Grünwald-Giemsa method.

Chapter III deals with histamine in normal skin. Species variations in tissue histamine have been known for several years. The author investigated skin histamine levels in man, pig, rabbit, guinea-pig, rat and mouse. Skin histamine was found high in mouse and rat, less in man and pig, while the guinea-pig and the rabbit had relatively low skin histamine.

Other workers have found striking regional differences in skin histamine in various animals and human autopsy material. The present author reports on a study of regional variations in adult human skin from patients without skin diseases. The same pattern was found as described in experimental animals. The highest levels occurred on the eyelids, cheeks and upper lip. High values were also found in other areas of the head, neck and dorsum of the hands. Individual variations were great. The influence of age was studied in human fetal skin and adult human skin. The investigations revealed a rise in skin histamine with increasing fetal age. Adult human skin showed a moderate decrease in histamine content with advancing age. No significant differences were found between skin histamine of women and men. When skin samples are split into an inner and an outer layer, previous investigators have found that man, cat and dog have their main concentration of histamine in the inner layer, while mouse, guinea-pig and rabbit have almost even concentrations in the inner and outer layer of skin. The

author studied the same matter in pig and found the highest concentration of histamine in the outer layer.

It was concluded that certain important factors should be considered when studying skin histamine: species differences, regional variations, the age of the individual, individual variations, whether the samples consist of whole skin or only a part of the skin, and the method of tissue extraction employed.

The relationship between skin histamine and mast cells was studied in human fetal skin, in *urticaria pigmentosa*, in experimental skin tumours of mice, in full thickness skin grafts of pigs and rats, and in fresh granulation tissue of pigs. The results are in agreement with the idea that the histamine value of skin reflects its mast cell population and that mast cells are sources of cell-bound skin histamine.

Skin histamine and mastocytosis was studied in *chapter IV*. In *urticaria pigmentosa*, the skin manifestation of human mastocytosis, the highest values were found in the pigmented skin of the youngest individuals. The differences in histamine content of pigmented skin between the infants and the patients more than twelve years old was remarkably high. It contrasted with the small differences found between the histamine levels of the non-pigmented skin of the two age groups. A high skin histamine value was also found in a mouse with a transplantable mastocytoma located to the mesentery.

Skin histamine in growth and repair is referred to in *chapter V*. It has been suggested that tissue edema initiates any process of growth, regeneration and repair of connective tissue. As histamine induces increase of vascular permeability, thus producing a tissue edema, it may be of importance under such conditions. The edema however may also be primary and responsible for the release of histamine. Besides histamine may stimulate active transport processes across endothelial barriers.

As mentioned above, the investigations on human fetal skin revealed a rise in skin histamine with increasing fetal age. The investigations also demonstrated a high ratio of wet weight to dry defatted weight declining with increasing fetal age. Histamine did not appear in skin in larger quantities until the fifth fetal month.

The investigations on full thickness skin grafts of pigs and rats showed a considerable release of histamine in the graft during the first days after the operation. Simultaneously an edema appeared. In the fresh granulation tissue of the pig skin no measurable histamine was found.

Malignant growth is another type of rapid tissue growth. Histamine determinations on a limited amount of experimental skin tumours in mice showed a lower average value for skin carcinomas than for non-malignant skin papillomas.

Considering the importance of histamine and mast cells in tissue repair and growth, we may conclude that histamine contents were low, and identifiable mast cells were few in skin during rapid tissue growth. It is proposed that the demand for histamine is so considerable, that a storage of cell-bound histamine in skin can scarcely take place. Evidence, however, is not yet complete.

Between the discovery of histamine in 1907 and the demonstration in 1932 of histamine release from the tissues of the sensitized animal on contact with the antigen, the concept of the participation of histamine in immediate allergic reactions was based on analogies between its pharmacological effects and those typical of anaphylaxis. Since then, several workers have published evidence of histamine release in connection with immediate allergic reactions of the skin. This subject is referred to in *chapter VI*.

Chapter VII relates to histamine and delayed skin reactions. Analyses of skin histamine were made in experimental allergic contact dermatitis (positive allergic patch tests) and normal skin of patients with contact dermatitis. Besides, a series of skin samples from induced primary irritant dermatitis was examined. No significant alterations were found in primary irritant dermatitis or in the initial stages of allergic reactions. After 24 and 48 hours, however, a marked rise in skin histamine was observed in the allergic patch tests. Although histamine accumulation also can be found in non-allergic inflammatory skin reactions, the investigations here presented indicate that in patch tests a significantly higher histamine level is associated with allergic response in contrast to non-allergic reaction. This observation has bearing upon the differential diagnosis between allergic and primary irritant skin reactions in patch tests. The results also suggest that histamine may have a part to play in delayed allergic reactions of the skin.

Atopic dermatitis, urticaria, and erythema multiforme have all been associated with allergy. Urticaria may occasionally be considered an immediate allergic reaction of the skin. In atopic dermatitis and erythema multiforme no definite proof has been presented of antigen-antibody reactions being the cause of the skin manifestations. Therefore, these diseases have been placed under the more neutral heading of itching dermatoses. Skin histamine in atopic dermatitis, urticaria, erythema multiforme, and bullous pemphigoid is the topic of *chapter VIII*. Other workers have found an increased skin histamine level in atopic dermatitis. In the studies of the author, the histamine content of wealing skin in spontaneous urticaria and urticaria factitia was lower than that of corresponding normal skin, thus indicating a release of histamine. These results are in agreement with the fundamental studies by *Lewis & Grant* (1924) on the wealing response. The etiology and pathogenesis of erythema multiforme is still obscure. A marked rise in skin histamine, together with the results of histologic and electron

microscopic studies by other workers, and the results of clinical treatment with antihistamine drugs indicate that histamine may play a part in this disease. In bullous pemphigoid the number of patients studied was too small to allow any conclusions. More generally regarding histamine and itching it is concluded that although histamine, when introduced into the skin, produces itching, and it possibly plays a part in some itching dermatoses, its role in symptomatic itching is not elucidated.

Some considerations concerning histamine and eosinophils are mentioned in *chapter IX*. For many years it was believed that eosinophils were the chief carriers of histamine in human blood. More recently, however, it has been shown that the basophil leukocyte, despite its scarcity is responsible for about fifty per cent of blood histamine. Lately the basophil-eosinophil and the mast cell-eosinophil relationships have been studied by several workers. The leukocytic response to aseptic inflammation in urticaria pigmentosa was studied. An extremely high percentage of eosinophils appeared in early "skin window" preparations. It was suggested that mechanical trauma elicited mast cell degranulation and histamine release, and that the release of histamine caused emigration of eosinophils. Histamine has been reported to have a chemotactic effect upon eosinophils. Eosinophils are by some investigators considered as wandering cells capable of inactivating histamine on demand. In severe urticaria eosinopenia is common. Repeated injections of a histamine liberator, polymyxin B, gave rise to an increasing eosinophilia in patients with chronic urticaria. The majority of patients seemed to derive a clinical benefit from the treatment, maybe on account of the eosinophilia.

Glucocorticoids delay the development of all the elements of the connective tissue. *Chapter X* concerns hormones and skin histamine, particularly the influence of glucocorticoids on histamine in human skin. This was studied using therapeutic doses of betamethasone. Oral administration of daily doses through two weeks markedly reduced the average histamine content of grossly normal skin in patients suffering from various dermatological diseases. Cortisone and other glucocorticoids have been found by other workers to exert pronounced decreasing effects on histamine formation in the rat. It is suggested that the lowered histamine content of skin may explain in part some of the therapeutic effects of glucocorticoids on human skin. Probably some of the side-effects, as for instance delayed wound healing and growth inhibition, may be explained through the same influence upon histamine. The influence of adrenalin, the thyrotropic hormone, thyroxin, and sex hormones upon skin histamine still remains to be elucidated.

Histamine liberators and their effects on skin histamine are described in *chapter XI*. The local and systemic effect of polymyxin B, a basic poly-

peptide, normally in use as an antibiotic, was studied by the author. Polymyxin B injected intradermally markedly reduced skin histamine at the site of the injection in 9 patients suffering from various skin diseases, when investigated one hour after the injection. The effect, however, seems to be short lasting. No significant alteration was found in 10 other patients four days after a similar injection. The systemic effects of polymyxin B were in agreement with its quality as a histamine liberator. The systemic effects were more pronounced in patients with chronic urticaria than in patients suffering from varicose ulcers, psoriasis, cutis hyperelastica, or condylomata. Repeated injections of polymyxin B, as mentioned above, produced a pronounced eosinophilia in patients with chronic urticaria. The dose of polymyxin B, used by the author, did not when given intramuscularly generally deplete the skin of histamine.

The general conclusions based upon a review of the literature and the results of the authors investigations are given in *chapter XII*. It is concluded that the studies on skin histamine levels and changes in skin histamine have indicated that histamine may have a part to play within tissue growth, regeneration and repair. Evidence is not complete, but this should be the trail to be followed when seeking the possible physiological importance of histamine in skin, as well as in connective tissue in general. It is believed that histamine is of pathogenetic importance in urticaria and for the wealing of urticaria pigmentosa. Whether histamine is of importance in the other skin diseases investigated, in particular in delayed allergic reactions, is still obscure. It is stated that we to-day have tools for further investigations: both corticosteroids and histamine liberators influence skin histamine. It is mentioned that whether or not eosinophils are capable of inactivating histamine has not been proved, but that the results obtained make it desirable to continue work along these lines. It is also suggested that work should be employed to investigate the presence and behaviour of other mast cell substances in skin, and that work on skin histamine should be continued, possibly including investigations concerning histamine metabolism.

Chapter XIV

Summary in Danish

Den stadig øgede forskning inden for dermatologien og forståelsen af histamins vigtige rolle som et ødemfremkaldende stof har nødvendiggjort yderligere undersøgelser over histamin i normal og patologisk forandret hud. Som nævnt i *indledningen* er det blevet muligt ved hjælp af den spektrofluorometriske metode at måle histaminindholdet i små hudbiopsier.

Formålet med forfatterens undersøgelser har været at bestemme histaminindholdet i normal og patologisk forandret hud, at forsøge at belyse betydningen af forandringer i hudens histaminindhold og at give en oversigt over histamins rolle i huden.

I *kapitel I* gives der en kort oversigt over kemien og omsætningen af histamin i organismen. Histamin dannes ved enzymatisk dekarboksylering af histidin og forekommer i tre former i organismen: som frit histamin, som bunden histamin og som konjugeret histamin (acetylhistamin). De to førstnævnte former er de vigtigste. Endskønt det er som frit histamin, at dette stof udøver sin virkning, findes kun små mængder frit histamin intercellulært i vævsvæskerne. Så langt den største mængde findes cellulært bundet, hovedsagelig i mastcellerne. Den nærmere mekanisme ved bindingen er ikke kendt. Efter dannelse og binding frigøres histamin under normale forhold langsomt fra vævene. Histaminfrigørelse kan også finde sted hastigt under påvirkning af forskellige stimuli, kemiske såvel som fysiske. Histamin udskilles i urinen i uomdannet form eller omdannet til metylhistamin, imidazolyleddikesyre, metylimidazolyleddikesyre eller acetylhistamin. Metylering af histamin efterfulgt af en oxydering af det dannede metylhistamin og oxydering af histamin er de vigtigste metaboliske processer.

Kapitel II beskriver de metoder, der er anvendt af forfatteren, fortrinsvis den spektrofluorometriske metode til histaminbestemmelse. De indledende forsøg, som er udført for at bedømme metodens pålidelighed refereres. Så godt som alle bestemmelser er foretaget på affedt og tørret hud. Affedtingen er foretaget for at undgå mulige fejl på grund af tilstedeværende talgkirtler eller eventuelle rester af subkutan fedtvæv. En tørring af vævet

og beregning ud fra tørret vægt er vigtig, når man bestemmer et ødemfremkaldende stof som histamin. De fleste hudbiopsier er taget i lokalanæstesi. Lokalanæstesiens indflydelse på bestemmelserne er undersøgt og fundet betydningsløs ved den af forfatteren anvendte teknik. Genfindelsen af histamin var gennemsnitlig 9,62 % med yderværdierne 86 og 110 %. Det svage led i alle bestemmelser af histamin i væv er adskillelsen af histamin fra histidin, derfor blev genfindelsen af histidin undersøgt. Den blev fundet til 0 %. Som støtte for, at den målte fluorescens hidrører fra histamin, må fremhæves, at excitations- og fluorescenskurverne for reel histamin og målt histamin i hud blev undersøgt og fundet identiske. Nogle opløselighedskaraktistika for reel histamin og målt histamin i hud blev ligeledes undersøgt og fundet praktisk talt ens. Det konkluderes, at man ved hjælp af spektrofluorometri kan bestemme hudens histaminindhold enkelt, nøjagtigt og tilstrækkelig specifikt.

Ved de histologiske undersøgelser af huden er anvendt den metakromatiske farvning med toluidinblåt efter forudgående fixation af vævet i basisk blyacetat. Leukocytf forholdene i celleemigrationen ved en aseptisk inflammation hos patienter med urticaria pigmentosa blev undersøgt ved hjælp af "hudvinduer". Hudvinduepræparaterne farvedes efter May-Grünwald-Giemsas metode.

Kapitel III beskæftiger sig med histamin i normal hud. Artsvariationer i vævshistamin har været kendt i adskillige år. Forfatteren har undersøgt hudens histaminindhold hos mennesket, grisen, kaninen, marsvinet, rotten og musen. Hudens histaminindhold fandtes højt hos musen og rotten, mindre hos mennesket og grisen, medens marsvinet og kaninen havde relativt lave histaminværdier i huden.

Andre har fundet betydelige regionsforskelle i hudens histaminindhold hos forskellige dyr og i sektionmateriale. Forfatteren har gennemført en undersøgelse af de regionale variationer i human hud fra voksne patienter uden hudsygdomme. De højeste værdier fandtes i øjenlåg, kinder og overlæbe. Høje værdier fandtes også i andre områder på hovedet, i nakken og på håndryggen. Variationerne fra individ til individ var store. Alderens indflydelse undersøgtes i human hud fra fostre og voksne individer. Undersøgelserne viste, at hudens histaminindhold tiltager med stigende fosteralder. Hud fra voksne individer frembød et moderat fald i histaminindhold med alderen. Der fandtes ingen signifikant forskel på hud-histamin værdierne hos mænd og kvinder. Efter at have delt hudbiopsier i et ydre lag og et indre lag, har tidligere forfattere fundet histaminkoncentrationen højest i det ydre lag hos mennesket, hunden og katten. Rotten har den højeste koncentration i det inderste lag, medens musen, marsvinet og kaninen har næsten samme koncentration i hudens yderste og inderste lag. Forfatteren undersøgte det samme forhold hos grisen og fandt den højeste histaminkoncentration i det yderste lag.

Det konkluderes at visse faktorer er af betydning ved undersøgelser over hudens histaminindhold: Artsvariationer, regionale forskelle, individets alder, variationer fra individ til individ, om prøverne består af fuld hud eller kun af en del af huden og metoden, der er anvendt til histaminekstraktion fra vævsprøven.

Relationen mellem hud-histamin og mastceller undersøgtes i human fosterhud, i hud fra patienter med urticaria pigmentosa, i experimentelle hudtumorer hos mus, i fuld hudstransplantater hos grise og rotter og i friskt granulationsvæv hos grise. Resultaterne stemmer overens med den opfattelse, at hudens histaminværdi giver et billede af dens indhold af mastceller, og at mastcellerne er hjemstedet for den i cellerne bundne histamin.

Undersøgelser over hud-histamin og mastocytose beskrives i *kapitel IV*. Ved urticaria pigmentosa, mastocytosens hudmanifestation hos mennesket, fandtes de højeste værdier i pigmenteret hud fra de yngste individer. Der fandtes en betydelig forskel på histaminindholdet i pigmenteret hud fra småbørn og fra patienter der var over 12 år gamle. I modsætning hertil var der meget ringe forskel på histaminværdierne fra de ikke pigmenterede områder af huden fra de to aldersgrupper. Et højt hud-histamin-indhold fandtes også hos en mus med et transplantabelt mastocytom i mesenteriet.

Den hypotese har været opstillet, at alle vækst-, regenerations- og helingsprocesser i bindevævet forudgås af ødem. Histamin kan have betydning ved disse processer, da det øger karrenes permeabilitet og således fremkalder ødem i vævene. Ødemet kan dog også være primært og årsag til en sekundær histaminfrigørelse. Histamin er sandsynligvis af betydning for transporten gennem endotelbarrierer. Hudens histamin under vækst og heling omtales i *kapitel V*.

Som ovenfor nævnt afslørede undersøgelserne af fosterhud en stigning i histaminindholdet med stigende fosteralder. Undersøgelserne viste også, at forholdet mellem våd vægt og tørret affedt vægt var højt, men aftog med stigende fosteralder. Histamin fandtes ikke i huden i større mængder før femte fostermåned.

Undersøgelserne af fuld hudstransplantater hos grise og rotter viste en betydelig histaminfrigørelse i transplantatet de første dage efter operationen. Samtidig optrådte der ødem. I det friske granulationsvæv fra grischud fandtes der ikke målelige mængder histamin.

Maligne tumorer er en anden type af væv i hurtig vækst. Histaminbestemmelser på et lille antal eksperimentelle hudtumorer hos mus viste en lavere gennemsnitsværdi for hudcarcinomer end for ikke maligne hudpapillomer.

Når histamins betydning for vævsvækst og heling tages i betragtning, må det understreges, at histaminindholdet var lavt og de identificerbare mastceller kun få i hud i hurtig vækst. Som en mulig forklaring herpå an-

føres, at histaminforbruget kan være så stort, at en samtidig opbygning af cellebunden histamin ikke kan finde sted.

Fra histamins opdagelse i 1907 til det i 1932 påvistes, at histamin frigøres i vævene hos det sensibiliserede dyr ved kontakt med antigen, baseredes opfattelsen af histamins deltagelse i allergiske reaktioner af den øjeblikkelige type på analogi mellem dens farmakologiske virkning og de typiske symptomer ved anafylaksi. Siden da har flere forfattere bevist, at histamin frigøres ved allergiske reaktioner af den øjeblikkelige type. Dette emne omtales i *kapitel VI*.

Kapitel VII omhandler histamin og senreaktioner i hud. Histaminanalyser foretoges på positive allergiske lappeprøver (eksperimentel allergisk kontaktdermatitis) og normal hud fra patienter med kontaktdermatitis (kontakteczem). Desuden undersøgtes histaminindholdet i hud med primært irritative (toksiske) reaktioner. Der fandtes ingen signifikante ændringer i hudens histaminindhold ved de primært toksiske reaktioner, eller i begyndelsesstadiet af de allergiske reaktioner. Efter fireogtyve og otteogfyrre timer observeredes imidlertid en betydelig stigning i histaminindholdet i de allergiske lappeprøver. Skønt en akkumulation af histamin også kan findes ved ikke allergiske betændelsesreaktioner i huden, peger de her beskrevne undersøgelser henimod, at en høj histaminværdi i en lappeprøve antyder en allergisk reaktion i modsætning til en ikke allergisk (toksisk) reaktion. Denne observation kan have betydning ved differentialdiagnosen mellem allergiske og primært irritative reaktioner i lappeprøver. Resultaterne viser også, at histamin muligvis spiller en rolle ved allergiske senreaktioner i huden.

Prurigo Besnier, urticaria og erythema multiforme har alle været sat i forbindelse med allergi. Urticaria kan i nogle tilfælde regnes for at være en allergisk hudreaktion af den øjeblikkelige type. For prurigo Besnier's og erythema multiformes vedkommende er der endnu ikke ført afgørende bevis for, at antigen-antistofreaktioner er årsagen til hudmanifestationerne. Derfor er disse sygdomme anbragt i den mere neutrale gruppe: kløende hudlidelser. Hud-histamin ved prurigo Besnier, urticaria, erythema multiforme og bulløst pemphigoid er emnet for *kapitel VIII*. Andre forfattere har fundet et forhøjet histaminniveau ved prurigo Besnier. I forfatterens undersøgelser var histaminindholdet i urticariaelementer ved spontan urticaria og urticaria factitia lavere end i tilsvarende normal hud, hvilket kan tydes som udtryk for histaminfrigørelse. Dette stemmer overens med resultaterne af de grundlæggende undersøgelser over urticariaelementers dannelse udført af *Lewis & Grant* (1924). Erythema multiformes ætiologi og patogenese er endnu ikke klarlagt. En tydelig stigning i hudens histaminindhold sammen med resultaterne af andre forfatteres histologiske og elektronmikroskopiske undersøgelser og resultaterne af klinisk behandling med anti-histaminica giver grund til at antage, at histamin spiller en rolle ved denne

sygdom. Antallet af undersøgte patienter med bulløst pemphigoid var for ringe til at tillade nogen konklusioner. Med hensyn til et eventuelt sammenhæng mellem histamin og kløe må det bemærkes, at til trods for at histamin injiceret i huden fremkalder kløe, og skønt histamin muligvis spiller en rolle ved visse kløende hudlidelser, er dets betydning for symptomatisk kløe ukendt.

Nogle overvejelser over histamin og eosinofile leukocyter omtales i *kapitel IX*. I mange år mente man, at de eosinofile leukocyter var de vigtigste bærere af histamin i blodet hos mennesket. For nylig er det imidlertid bevist, at de basofile leukocyter til trods for deres ringe antal indeholder omkring 50 % af blodets histamin. I den seneste tid har adskillige forfattere studeret forholdet mellem basofile og eosinofile leukocyter og mellem mastceller og eosinofile leukocyter. Leukocytforholdene i celleemigration ved urticaria pigmentosa omtales. Et meget stort antal eosinofile leukocyter optrådte i tidlige hudvinduepræparater. Som mulig forklaring herpå foreslås, at det mekaniske traume har forårsaget mastcelledegranulering og histaminfrigørelse, hvorefter histaminfrigørelsen bevirker en udvandring af eosinofile leukocyter. Andre undersøgere har vist, at histamin har en kemotaktisk effect på eosinofile leukocyter. Disse opfattes af nogle forfattere som vandrende celler, der er i stand til at inaktivere histamin, når der er behov herfor. Ved kraftig urticaria er eosinopeni almindelig. Gentagne injektioner af en histaminliberator, polymyxin B, forårsagede en tiltagende eosinofili hos patienter med kronisk urticaria. Hos de fleste patienter syntes behandlingen at bevirke en klinisk bedring, muligvis forårsaget af eosinofilien.

Glukokortikoider forsinker udviklingen af alle bindevævs elementer. *Kapitel X* omhandler hormoner og hudhistamin med særlig henblik på glukokortikoiders indflydelse på histamin i human hud. Ved undersøgelserne anvendtes terapeutiske doser af betamethasone (flubenisolum NFN). Peroral indgift af daglige doser formindskede i løbet af to uger signifikant det gennemsnitlige histaminindhold i normal hud fra patienter med forskellige hudsygdomme. Kortison og andre glukokortikoider er af andre forfattere fundet at have en udtalt hæmmende effekt på histamindannelsen hos rotter. Det anføres som en mulighed, at det nedsatte histaminindhold til dels kan forklare nogle af glukokortikoidernes terapeutiske virkninger på human hud. Muligvis kan også nogle af bivirkningerne, som for eksempel forsinket sårheling og nedsat vækst forklares gennem den samme indflydelse af histamin. Virkningen af adrenalin, det thyreotrope hormon, thyroksin og kønshormoner på hudens histamin er endnu ikke klarlagt.

Histaminliberatorer og deres effekt på hudens histamin beskrives i *kapitel XI*. De lokale og universelle virkninger af polymyxin B, et basisk polypeptid, sædvanligvis anvendt som antibioticum, undersøgt af forfatteren. Polymyxin B injiceret intradermalt hos ni patienter med forskellige hud-

sygdomme, bevirkede en tydelig reduktion af hudens histaminindhold på injektionsstedet een time efter injektionen. Virkningen syntes dog kun at være af kort varighed. Ingen signifikante ændringer fandtes hos ti andre patienter fire dage efter en lignende injektion. Polymyxin B's universelle virkninger stemte overens med dets egenskab som histaminliberator. De universelle virkninger var mere udtalte hos patienter med kronisk urticaria end hos patienter lidende af varikøse ulcera, psoriasis, cutis hyperelastica eller kondylomer. Gentagne injektioner af polymyxin B fremkaldte, som ovenfor nævnt, en udtalt eosinofili hos patienter med kronisk urticaria. Den af forfatteren anvendte polymyxindosis bevirkede ikke ved intramuskulær injektion nogen almen nedsættelse af histamin i huden.

Konklusionerne af en gennemgang af litteraturen sammenholdt med resultaterne af forfatterens undersøgelser behandles i *kapitel XII*. Det konkluderes, at undersøgelserne over histaminindholdet i huden og forandringerne heri tyder på, at histamin spiller en rolle ved vævsvækst, regeneration og heling. Der er ikke ført endeligt bevis herfor, men det gøres gældende, at det må være her, man skal søge histamins mulige fysiologiske rolle i huden og i bindevævet i almindelighed. Det formodes, at histamin er af betydning for patogenesen ved urticaria og for den traumatisk betingede hævelse i huden ved urticaria pigmentosa. Om histamin er af betydning for de andre i undersøgelsen medtagne hudsygdomme er endnu uopklaret. Det fastslås, at vi idag er i besiddelse af midler til yderligere studier, idet både kortikosteroider og histaminliberatorer influerer på hudens histaminindhold. Det omtales, at det endnu ikke er bevist, hvorvidt de eosinofile leukocyter er i stand til at inaktivere histamin, men at de opnåede resultater opmuntrer til fortsatte studier heraf. Der foreslås endvidere undersøgelser af de øvrige i mastcellerne indeholdte kemiske stoffer, og at studierne over hudens histamin fortsættes, muligvis medinddragende undersøgelser over histaminomsætningen.

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